THE INFLUENCE OF GROUPS IN THE MOLECULE OF

3'-KETO - 2:3 - DIHYDROENZ - 1:4 - THIAZINE

ON ITS

"ANTHELMINTIC" PROPERTIES

by

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INTRODUCTION

A number of investigations have been carried out on the relationship between chemical constitution and anthelmintic potency but the results so far obtained have been rather conflicting and at present it would appear that much work has still to be done in this field. It is only within the homologous series of chemical compounds that some correlation has been found between the effect on a particular species of worm and the number of certain groups in the molecule.

Baldwin (Brit. J. Pharmacol., 1948, 2, 91) attributes the conflicting results to the method of in vitro testing used. Trendelenberg (Arch. exp. Path. Pharmak., 1916, 72, 190) and the early workers used annelids but this was condemned by Lamson and Ward (Science, 1936, 84, 293) who tested many widely different chemical substances on both earthworm and pig Ascaris and found that the results showed no correlation of action.

The use of intact specimens of pig Ascaris by Lamson was a notable advance in technique. Lamson, however, noted that pig Ascaris was extremely resistant to many drugs, a fact also noted by von Schroeder (Arch. exp. Path. Pharmak., 1885, 19, 290). This method however, did not give any indication as to the mode of action of the compound under test. Baldwin overcame this difficulty by using small
tied-off neuromuscular preparations of pig *Ascaris* with an intact cuticular layer. Unlike earthworm and leech preparations, these were found to be unreactive to any of a large number of compounds known to be devoid of anthelmintic properties but possessing powerful physiological or pharmacological activity of other kinds, and to respond to most compounds known to possess anthelmintic potency. Furthermore Baldwin found that the normal test preparations of *Ascaris* failed to respond to acetylcholine at concentrations as high as 1 in 5000, but that preparations where the muscle was directly exposed to the compound were stimulated at concentrations of the order of $1:10^5 - 1:10^6$ thus it appeared that the failure of the normal preparations to respond to acetylcholine was not due to the insensitivity of the muscle to the compound but to the impermeability of the cuticle to the compound. Further evidence of the highly selective permeability of the cuticle of pig *Ascaris* has been forwarded by Trim (Parasitol. 1944, 22, 2)9).

A satisfactory ascaricide must therefore possess at least two attributes namely -

(a) It must be capable of penetrating the nematode cuticle

(b) having penetrated it must have a deleterious action upon the nematode tissues.

Hence a sound in *vitro* method for the detection and measurement of ascaricide activity should be
based on material of nematode origin with an intact cuticular layer and not on annelid material which does not possess this cuticular barrier.

One of the best known anthelmintics is Santonin, a drug which has been used in practice for a very long time.

At the present time what group or radical is responsible for its outstanding activity is not known but many theories have been put forward, the earliest of these being due to Trendelenberg who attributed the activity to the presence of the lactone ring. Trendelenberg based his theory on the fact that santonin, santonin oxime and tetrahydrosantonin, all of which contain the lactone ring, had a marked activity while santoninic acid in which the ring is open was inert. In support of his theory he stated that other lactones e.g. pilocarpine and coumarin similarly lost their effect when the ring was opened. Trendelenberg's theory was further supported by Lautenschläger (Ber. dtsch. Pharm. Ges., 1921, 31, 279), von Oettingen (J. Pharmacol., 1929, 36, 335), Gluschke (Arch. wiss. prakt. Tierheilk., 1932, 65, 201) and Rosenmund and Schapiro (Arch. Pharm., Berl., 1934, 272, 313). They prepared and tested a large number of lactones some of which they claimed to be more active than santonin itself.

In 1921 Oshika (Acta Schol. med. Univ. imp. Kioto, 1921, 4, 421) came to the conclusion that the
activity of santonin in the case of earthworm muscle was not wholly due to the lactone ring because he found the ethyl esters of santoninic and santonic acids both active, the corresponding free acids being inert.

It was also noted that lactonisation was not possible in either santonic or santonous acids a fact which led Caius and Mhaskar (Ind. J. med. Res. 1923, 11, 377) to the conclusion that the active centre was the ketonic group of the unsaturated ring. Baldwin found that aliphatic aromatic ketones, e.g. benzylidene acetone, and a group of related ketones possessed appreciable anthelmintic potency, a fact which seemed to point to the ketonic group.

It would therefore appear that the activity of santonin is due partly to the ketonic group and partly to the lactonic structure. The outstanding anthelmintic potency being derived from the simultaneous presence of both groups and from the unique manner in which they are combined rather than from either alone.

An examination of all the active compounds of santonin (I) leads to an interesting observation namely that, with one exception, santoninic acid, all the compounds have three structural features in common.

(a) an intact \( \gamma \) - lactone ring
(b) a double bond at position 7
(c) an angular methyl group at position 10.

\[
\begin{align*}
\text{(I)} & \\
\end{align*}
\]

The inert compounds have one or more of these characters absent.

The strong anthelmintic potency of santonin influenced Gluschke to prepare two closely related compounds namely the syntonins

\[
\begin{align*}
\text{Syntonins} & \\
\end{align*}
\]

which he claimed to equal or surpass santonin in activity when tested on earthworm muscle. Baldwin, however, found alantolactone (II) a substance more closely allied to santonin than the syntonins, to be totally inert when tested at a concentration of 1 in 2000.

\[
\begin{align*}
\text{(II)} & \\
\end{align*}
\]

Anthelmintic activity is in no way confined to ketonic groups and lactone structures, many other groups have been found to have an effect on this activity.
Iautenschl"uger (loc. cit.) while working on γ-lactones found that the introduction of a phenyl radical greatly increased the activity. He claimed that γ-phenylbutyrolactone and phenylparaconic acid lactone were about as half as active as santonin upon earthworm preparations, whilst phthalide (α:β-benzbutyrolactone) was as active as santonin itself. Following up this work of Lautenschl"uger's, Baldwin examined chromone (III), 2-coumaranone (IV) and 3-coumaranone (V) together with some of their derivatives.

He found that in none of these fused ring compounds was there any activity approaching that of santonin or even that of phenylbutyrolactone. Baldwin, therefore concluded that compounds with separated rings have greater activity than those with fused rings. Further evidence was found in the case of the pyridines, 4-benzylpyridine being much more active than pyridine itself. Similar results were found with the phenols but in the case of the thiazoles there was little to choose between fused and separated rings. Since the introduction of a benzene ring gave
an increase in activity the next step was to explore the effect of a second ring. Baldwin prepared a series of dipyridyls and found these the most active he had encountered amongst pyridine derivatives, 2-2' - dipyridyl showed an activity comparable with that of santonin and was the most active of the four dipyridyls prepared. The high potency appeared to be associated with the 2-2' - linkage because a fall occurred when this was moved to the 2-3' - position. Following up on this idea Baldwin prepared compounds containing pairs of nitrogen atoms linked to adjacent carbon atoms and only found activity in the phenanthrolines, of these the 4:5 - phenanthroline (VI) showed activity equal to that of 2:2' - dipyridyl, the other two being feebly active or inert.

Baldwin therefore concluded that the high activity was due to the bond system which was common to the two most active compounds.

Such compounds would appear to form an ideal starting point for new anthelmintics but unfortunately it has been pointed out by Harwood (Proc. Soc. exp. Biol., N.Y., 1934, 32, 131) that successful anthelmintic drugs for treatment of intestinal infestations have melting-points below 80°C and are
only sparingly soluble in water (1 in 1000 or less). These new substances are too soluble to be of much value in the removal of intestinal nematodes. It might however be possible to produce similar compounds which are insoluble, and therefore valuable in intestinal infestations.

Anthelmintic activity has been found in many cases to vary with the nature and length of the side-chain. Baldwin (loc. cit. 95) found that the introduction of alkyloxy radicals into position 4 of benzylidene acetone but not of acetophenone increased the activity, thus it would appear that the unsaturated side-chain carries greater anthelmintic potentialities than the saturated side-chain. Anthelmintic activity was also found to vary with the length of the chain, a maximum activity is reached whereupon increase in the length decreases the activity. Similar results were obtained by Lamson, Brown and Ward (J. Pharmacol., 1935, 53, 198) on testing a series of 4-n-alkylresorcinols. They found the activity to increase through butyl, amyl and hexyl whilst a fall occurs with heptyl, the activity being practically absent above duodecylresorcinol.

Normal chains were found by Lamson, Brown, Stroughton, Harwood, Baltzly and Bass (loc. cit. 218) to be more effective than branched chains with the same number of carbon atoms.
Many phenols are known to possess anthelmintic activity e.g. thymol, β- naphthol and hexylresorcinol in clinical medicine and veterinary science. Attempts have been made by Baldwin (J. Pharmacol., 1948, 2, 102) to find new active derivatives of active phenols. The carbamates are the most promising found so far and suggested an investigation of some unsaturated amides and substituted ureas none of which, however, proved of interest.

The position regarding phenols Baldwin summed up as follows.

1. Anthelmintic activity in phenols and their carbamates has been confirmed.

2. The position of the hydroxyl (OH) group has different effects in different chemical compounds.

3. Phenols with independent ring systems are more active than those with condensed rings.

4. The carbamate, 2-hydroxydiphenylcarbamate shows considerable promise.

Since thiazoles have been shown to be important as antibiotics in the chemotherapy of bacterial diseases it was thought that they might be of some anthelmintic value. 2- Amino - thiazole proved a good starting point but was inactive, the activity being increased on the introduction of a phenyl radical. The thiazoles, however, proved disappointing, little or no effect on anthelmintic
potency being found.

Other groups which may have some effect on anthelmintic activity are the methyl and carboxyl groups. Von Oettingen (J. Pharmacol. 1929, 36, 335) found that the activity was greatly increased by the introduction of these two groups into the lactone ring. He also claimed an increase in activity on the introduction of a double bond.

Claims have also been made for the halogens. Wright and Schaffer (Amer. J. Hyg., 1932, 16, 325) state that anthelmintic potency increases with increase in halogen content of the molecule but that the water solubility is a modifying factor. They have, however, confined their attention to chlorine. It would be interesting to see if any change in anthelmintic effect is produced through the series fluorine, chlorine, bromine and iodine.

The compound chosen as starting point in this investigation was 3-keto-2:3-dihydrobenz-1:4-thiazine (VII).

This compound was chosen because it contains in its molecule, atoms and groups present in a number of
compounds which possess anthelmintic properties e.g. phenothiazine (VIII) which is used in veterinary medicine against round worms in the intestinal tract of swine, horses and ruminants.

\[
\begin{align*}
\text{S} & \\
\text{N} & \\
\text{(VIII)} & \\
\end{align*}
\]

3 - Keto - 2:3 - dihydrobenz - 1:4 - thiazine also contains a ketonic grouping which has been suggested by Baldwin and Moyle (J. exp. Biol., 1947, 23, 277) as partly contributing to the anthelmintic activity of Santonin (IX), a compound widely used against parasitic infestations.

\[
\begin{align*}
\text{CH}_3 & \\
\text{C=O} & \\
\text{(IX)} & \\
\end{align*}
\]

A further similarity to the benzthiazine derivative is to be found in filicic acid (X), the most important of the organic acids of Filix mas, which is prepared from the powdered rhizome of the male fern. Filix mas is used for the removal of tapeworm from human digestive tracts. Comparison of the structures of 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine and filicic acid show that both con-
tain the grouping - CO-CH₂ - which may be partly responsible for anthelmintic activity.

\[
\text{On consideration of these similarities in structure it was reasonable to expect that 3-keto - 2:3 - dihydrobenz - 1:4 - thiazine itself would show some anthelmintic activity. Furthermore various groups can be conveniently introduced into the molecule in the 6-position by way of the diazo-reaction on the 6-amino compound, which was easily prepared in the laboratory. By introducing a large variety of different groups in this way, a means was therefore provided of studying the effect of the individual groups on the anthelmintic activity of the molecule.}

3-Keto - 2:3 - dihydrobenz - 1:4 - thiazine was therefore ideally suited for the purpose of the investigation.

The compounds tested were all derivatives of the above, prepared by introducing various groups into the 6- and 6:7-positions in the molecule. The following atoms or groups were introduced:

\[-\text{NH}_2, -\text{NH}_2\cdot\text{HCl, CH}_2\text{CONH-}, \text{F, Cl, I, CNS, } -\text{N}_2, -\text{NO}_2, -\text{NO}, -\text{SH, H}_2\text{AsO}_3, -\text{H}_2\text{SbO}_3, -\text{HgCl, 6:7 - dimethoxy and 6:7 - dihydroxy. In addition the following com-}\]
pounds were also tested 6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine coupled with phloroglucinol and salicylic acid, and 6:6'-bis-(3-keto-2:3-dihydrobenz-1:4-thiazine). It will be seen that the groups introduced were varied in nature in order to determine the relationship, if any, between chemical constitution and anthelmintic potency.

The unsubstituted 3-keto-2:3-dihydrobenz-1:4-thiazine was prepared from o-nitrophenylthioglucollic acid, obtained by reducing di-o-nitrophenyl-disulphide to o-nitrophenylthiol by the method of Glaasz (Ber. dtsch. Ges. 1912, 45, 2427) and converting the thiol to o-nitrophenylthioglucollic acid (Glaasz, loc. cit. 749). The yield of o-nitrophenylthioglucollic acid was good but the reduction of this compound to 3-keto-2:3-dihydrobenz-1:4-thiazine with zinc dust and glacial acetic acid gave a poor yield, the maximum being obtained by intimately mixing the o-nitrophenylthioglucollic acid with the zinc dust prior to adding the glacial acetic acid. The zinc dust used must be reasonably free from oxide. Reduction with tin and concentrated hydrochloric acid gave much poorer yields.

The majority of the derivatives, many of which have been isolated for the first time, were prepared from 6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine by way of the diazo-reaction so
giving derivatives with the required group substituted in the 6 - position.

The 6 - amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine was prepared in good yield from 2:4 - dinitrophenylthioglycollic acid by reduction with granulated tin and concentrated hydrochloric acid. The 2:4 - dinitrophenylthioglycollic acid was easily obtained from thioglycollic acid and 2:4 - dinitrochlorobenzene.

In the halogen series of derivatives, the 6 - fluoro, 6 - chloro and 6 - iodo - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine were prepared without much difficulty, but the 6 - bromo - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine could not be isolated in an analytically pure state. Several attempts were made by means of the Sandmeyer reaction using the diazonium sulphate and cuprous bromide solution but all proved unsuccessful. Further unsuccessful attempts were made to isolate the 6 - bromo - derivative by decomposing the diazonium perbromide by heating in an open tube suspended in boiling bromobenzene vapour for several hours.

Attempts to prepare the 6 - hydroxy - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine by way of the diazo reaction failed because self-coupling probably occurred during decomposition of the diazonium solution. 3 - Keto - 2:3 - dihydro - 6:7 - dihydroxybenz - 1:4 - thiazine, however, was easily prepared from the corresponding dimethoxy - compound
by demethylation with constant boiling hydriodic acid. The 6:7 - dimethoxy - derivative although easily prepared involved a long synthesis. The starting material was catechol which was converted to veratrole by reaction with dimethyl sulphate. Veratrole on heating with chlorosulphonic acid gave veratrole - 4 - sulphonyl chloride which on reduction with zinc dust and concentrated hydrochloric acid gave 3:4 - dimethoxy phenylthiol, in turn converted to 3:4 - dimethoxyphenylthioglycollic acid by interaction with monochloroacetic acid. Nitration of 3:4 - dimethoxyphenylthioglycollic acid with nitric acid (D, 1.42) gave the 2 - nitro - derivative from which 3 - keto - 2:3 - dihydro - 6:7 - dimethoxybenz - 1:4 - thiazine was obtained on reduction with tin and concentrated hydrochloric acid.

Attempts were also made to prepare the 6 - cyano - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine by way of the Sandmeyer reaction, but an analytically pure product could not be obtained. The Grignard reaction was also investigated, both in ether and acetone medium, but the reaction did not take place in either case.

In recent years much work has been done in an attempt to find a suitable screening test for compounds thought to possess anthelmintic properties. Baldwin (Parasitol., 1943, 35, 89) evolved a method whereby quantitative estimates of the potency of
compounds could be quickly, if only rather approximately, obtained. "The final test of an anthelminthic" wrote Lamson and Brown (Amer. J. Hyg., 1936, 23, 85) "is in its administration to the host and the determination of the percentage of the parasites expelled".

The difficulty in carrying out a suitable test is in finding a laboratory animal in which a controllable degree of nematode infestation can be maintained, and even when such an animal is available, the procedure of testing a large number of new compounds, the pharmacological properties of which are unknown, involves a great deal of time, money and living material. This being the case Baldwin set about to establish a form of in vitro test by means of which preliminary trials could be rapidly conducted on new substances, from which the most promising could be selected for further investigation.

The choice of a suitable material had to be made and Baldwin decided upon a nematode preparation. It had to be remembered, that numerous anthelmintics act specifically upon nematodes and others specifically on trematodes, whilst others attack both groups. Baldwin therefore concerned himself with the action of various compounds upon nematodes as represented by the roundworm of the pig, *Ascaris lumbricoides*.

Kymographic analysis technique had been
adopted by Rico (C.R. Soc. Biol., Paris, 1926, 94 918, 921) but rejected as unsuitable for chemotherapeutic investigations by Lamson and Brown (loc. cit.). They developed a method using intact healthy specimens of pig Ascaris and tested some hundreds of compounds by this method in the course of which they discovered the nematocidal properties of hexylresorcinol. This method gave little indication of the mode of action of the compounds tested on the helminth, and when new compounds are under test the type of action produced by each member of a series of compounds should be distinguishable, in order that attempts can be made, by alteration of chemical structure, to bring about some desirable change in the activity. For this reason Baldwin decided upon developing the kymographic technique which is also more economical in living material and compound. Furthermore the activity of each preparation may be tested prior to addition of the compound.

The most suitable nematode material available was Ascaris lumbricoides which may be kept alive in the laboratory for as long as 10 to 11 days, although by this time they are in a state of extreme physiological decrepitude. If used within 48 hours of removal from the host they are in a physiological condition closely approximating to normal. A further reason for using Ascaris was the fact that anthelmintics known to act upon such
species do, as a rule, act upon other groups, including for example liver fluke, threadworms, hookworms and certain strongyles.

The preparations used by Baldwin were of the tied-off tubular fragments of the kind proposed by Rico, only female worms were used and, in general, no worm was used after being in the laboratory for 48 hours. Two preparations were made from each worm, an 'intermediate' preparation corresponding to a ganglion-free nerve muscle preparation and an 'anterior' preparation containing the 'nerve-ring' which is thought to discharge the functions of a primitive 'brain'. In the majority of the compounds tested both preparations were found to respond in the same manner, while some compounds caused paralysis of the anterior preparation without affecting the intermediate preparation. It was observed that the tracings obtained with anterior preparations were always more complex than those of the intermediate preparations, probably due to the presence of the nerve-ring giving the anterior preparation a greater versatility of movement. Baldwin preferred to use the intermediate preparation, because of its simplicity, when quantitative information was required.

When such information was required of the compound under test it was easily available if the compound was water soluble, because then the effective concentration could be easily measured. Most
anthelmintics have low solubility, but it was discovered that these compounds often formed emulsions containing one part in from 1000 to 10,000 parts or higher of the diluent. By taking advantage of the stabilising effect of wetting agents such as sodium glycocholate, these emulsions could be made stable for periods of from 30 minutes to several hours. On testing the activity of such emulsions it was found that an emulsion of the compound was far more active than a suspension of the same concentration, also that a 1:1000 emulsion was approximately twice as active as a 1:2000 emulsion. The wetting agent had no effect on the movement of the worm preparation at the concentration used to prepare the emulsion. It, however, could not be concluded that the effective concentration of a compound applied in the form of an emulsion was the same as that of a solution of similar concentration, so that an accurate comparison of a water soluble compound with an insoluble compound was not possible. It was however found that similar chemically constituted insoluble compounds could be compared, by the effects they produced, when applied to Ascaris preparations in the form of emulsions of similar concentrations.

Such an in vitro test using fragmentary preparations of nematodes or intact nematodes does have certain disadvantages. The test may detect anthelmintic potency with compounds which although
active themselves become inactivated when in contact with the digestive juices of the host. This type of compound was found to be extremely rare. A second disadvantage which could have been put forward was in the nature of the preparations. These, as used by Baldwin, were essentially nerve muscle preparations and could not be expected to respond to anthelmintics other than those which acted on the neuro-muscular apparatus of nematodes. Most of the anthelmintics in common use, however, were found to act upon the neuro-muscular tissues but the fact that other tissues could be affected cannot be completely overlooked. The main objection Baldwin saw in his method was that the alimentary canal became completely occluded by the ligatures, therefore compounds applied externally could only gain access to the tissues by penetration of the cuticle, which earlier workers claimed to be highly resistant. From the results of recent work it would appear that the impermeability of the cuticle may have been exaggerated and that successful anthelmintics are capable of penetration.

Baldwin, on consideration of the results he obtained using his method, concluded that the preparations gave responses of a specific nature to compounds which were not closely related, a similarity of reaction to closely related compounds and consistent results when a particular compound was
used. His method was capable of detecting anthelminthic potency in compounds which were known to possess such potency, whilst compounds with no known anthelminthic properties gave little or no response to his preparations.

Although the work carried out by Baldwin was mainly concerned with testing the general applicability of the method and not with the intention of making an exhaustive study of the various compounds used, it however brought to notice several interesting facts.

It was found, for example, that filix mas and pelletierine both used against tapeworm were inactive towards the Ascaris preparations. Further, evidence emphasising the unsuitability of annelinid preparations as test materials for the study of anthelminetics acting upon nematodes was also brought forward.

Baldwin also compared the relative activities of a series of anthelminthic compounds by determining the concentration required to produce paralysis within 20 - 30 minutes after addition of the compound. No strict comparison was possible by this method because some of the compounds were used as solutions whilst others were used as emulsions. Approximate quantitative data, can, however, be obtained and there is no doubt that the method does serve as a preliminary device whereby promising compounds can be selected from a large group of new
compounds.

An investigation similar to that of Baldwin has been carried out by Chance and Mansour (Brit. J. Pharmacol., 1949, 4, 7). They used the kymographic technique but studied the effect of compounds on the liver fluke (Fasciola hepatica), as a typical platyhelminth. The liver flukes used were obtained from the bile ducts of bovine livers and were dissected out within half an hour of the death of the host before the liver cooled to room temperature. Chance and Mansour found the bovine flukes to be more reliable than the flukes from sheep livers.

The compounds were either tested in solution or as emulsions, the concentration used in the first instance being 1 in 1000, if active at this concentration lower concentrations, depending on the response obtained, were then tried. The minimal effective concentration was judged, in most instances, by the disappearance of activity in 45 minutes after the addition of the compound. The absence of effect at 1 in 1000 on at least two preparations was taken to indicate that the compound was without activity.

The compounds were classified as stimulant, paralysant or lethal. Among the stimulant compounds was amphetamine sulphate which possessed high activity. This stimulant action gave Chance and Mansour the idea to add it to restore normal rhythmical activity to preparations where the move-
ment had been reversibly altered by the compound. This might occur after inactivation by paralysant compounds but no such observation would be expected after subjection to the action of a lethal compound. It was therefore decided to add amphetamine sulphate in a concentration of 1 in 5000 to the bath, in the absence of the test substance, whenever the activity of the preparation had been altered after 45 minutes. This they did by replacing the solution of the test compound by Ringer's solution containing 1 in 5000 amphetamine sulphate. By this means they were able to distinguish between compounds with paralysant action and compounds with a lethal action.

The results obtained with liver fluke are interesting. Among the compounds tested by Chance and Mansour the chlorinated hydrocarbons at concentrations of 1 in 5000 to 1 in 1000 were found to be lethal, phenothiazine was found to be inactive whilst the amines, lactones and strychnine were found to be stimulant. Other compounds were found to be stimulant at low and lethal at high concentrations, for example the halogenated hydrocarbons, \( \beta \)-cymene and \( \beta \)-naphthol. Phenylurethane and oil of chenopodium had a paralysant effect at low concentrations becoming lethal as the concentration was raised. It was also observed that compounds which had an effect on Ascaris affected liver flukes at lower concentrations, and that liver flukes were affected by compounds which did not affect Ascaris.
A further type of action was that of coumarin which was found to be stimulant to fluke and paralysant to Ascaris.

In order to ascertain if 3-keto-2:3-dihydrobenz-1:4-thiazine and derivatives, which had been prepared in the laboratory, had any anthelmintic potency they were each subjected to an in vitro test. The method of in vitro testing employed, with exception of a few minor modifications, was essentially that of Baldwin. The preparations used in the in vitro test were of two types (a) preparations of roundworm, from the pig (Ascaris lumbricoides) and (b) preparations of liver fluke (Fasciola hepatica). The Ascaris and liver fluke were obtained, fresh, from the City Abattoir at Slateford and transported to the Heriot-Watt College in Ringer's solution at 38°C contained in vacuum flasks.

Bovine and sheep flukes were used and both gave satisfactory results. This differs slightly from the findings of Chance and Mansour who preferred bovine flukes, since it was found that the latter showed better rhythmical movement.

The Ascaris preparations used were mainly of the intermediate type because they give the simpler tracings and are preferable when quantitative results are required.

Of the derivatives of 3-keto-2:3-dihydrobenz-1:4-thiazine tested, only two were soluble
in Ringer's solution so that for the most part emulsions were employed. The maximum concentration used was 1 in 1000 but in several cases only suspensions could be obtained at this concentration. The compound, if active, was tested at different dilutions till a concentration was found, which was the minimum required to produce an effect on the helminth preparation. Several compounds, which were inactive at a concentration of 1 in 4000, could not be tested at higher concentrations as suspensions were formed at 1 in 4000.

The compounds were classified as (a) stimulant, (b) paralysant or (c) lethal, type (b) and (c) being distinguished by the use of amphetamine sulphate solution (page 223) which, however, has no stimulant effect on Ascaris and therefore could not be used to distinguish between paralysant and lethal effects in this case. The only means of distinguishing between paralysant and lethal effects in the case of Ascaris being to transfer the preparation from the test solution to fresh Ringer's solution and watch carefully for signs of recovery.

The minimum concentration required to produce either a paralysant or lethal effect was determined and so referred to as the "Threshold Value". It was then found possible to arrange the various groups in the following order of potency:

\[
\text{Cl} > \text{NO}_2 > \text{I} > \text{NO}_3 > \text{dimethoxy} > \text{NH}_2 \cdot \text{HCl} > \text{unsubstituted}, \text{F} > \text{NH}_2, \text{CH}_3, \text{CONH}-
\]
CNS-, SH- and 6:7 - dihydroxy.

Owing to the insolubility of the remaining groups it was not possible to ascertain a definite Threshold Value. It would appear that the latter was less than 4000 in these cases.

A number of the benz - 1:4 - thiazine derivatives were tested against free living stages of Bursate nematodes in faecal cultures in the Department of Zoology at the University of Edinburgh. The results of these tests are given in the experimental part of the thesis.
Compounds isolated for the first time are marked with an asterisk.

**3 - KETO - 2:3 - DIHYDROBENZ - 1:4 - THIAZINE**

3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine was prepared according to the following series of reactions.

\[ 
\begin{align*}
\text{o-chloronitrobenzene} & \quad \text{HCl} \quad Na_2S_2 \quad \text{di-o-nitrophenyl disulphide} \\
\text{3-keto-2:3-dihydrobenz-1:4-} & \quad \text{HN dust + Haze} \quad \text{o-nitrophenylthioglycolic acid} \\
\text{Thiazine} & \quad \text{Cl.CH_2COOH} \quad \text{o-nitrophenylthiol.}
\end{align*}
\]

**Di-o-nitrophenyl disulphide**

Di-o-nitrophenyl disulphide was prepared according to the method described in Organic Syntheses Vol. 8 p.64. Sodium sulphide crystals (72g, 1.5 mol) and alcohol (300 c.c.) were heated together in a flask fitted with a reflux condenser on a steam bath until solution of the sulphide was effected. Finely ground sulphur (10g., 1.5 mol) was added and the heating continued till the sulphur dissolved when a brownish-red solution of sodium disulphide was obtained. o-Chloronitrobenzene
(63g, 2.0 mol) dissolved in alcohol (100 c.c.) was placed in a flask fitted with a reflux condenser and the sodium disulphide solution slowly added down the reflux to the o-chloronitrobenzene. When the reaction had abated somewhat the mixture was heated on a steam bath until it became amber coloured (3 hours). The reaction mixture was then cooled, filtered and the residue stirred thoroughly in 100 c.c. water to dissolve the sodium chloride formed in the reaction. The mixture was filtered, washed free from chloride, and the residue washed with 20 c.c. alcohol to remove any o-chloronitrobenzene. The product gave a melting-point 184 - 186°, melting point given is 192 - 195°, and was pure enough for the next stage in the synthesis.

Yield: --, 40.5 g, (66% theoretical)

**o-Nitrophenylthioglycollic acid**

Di-o-nitrophenyl disulphide (40g) was reduced to o-nitrophenylthiol by the method of Claasz (Ber. dtsch. chem. Ges., 1912, 45, 2427). The disulphide was mixed with glucose (28g) and the mixture warmed with alcohol (130 c.c.) on the steam bath. A solution of sodium hydroxide (22g) in water (40 c.c.) was slowly added, the flask being carefully shaken periodically. After a few minutes a vigorous reaction took place and the colour of the solution turned reddish-brown, when the sodium salt of the thiol was formed. The thiol was then converted to
o-nitrophenylthioglycollic acid as described by Claasz (loc. cit. 749). The reddish-brown solution was diluted with warm water (670 c.c.), stirred well, cooled and filtered. A solution of monochloracetic acid (28g.) in water (ca 130 c.c.) was neutralised with anhydrous sodium carbonate and the sodium monochloracetate solution added to the reddish-brown filtrate. The resulting mixture was heated on a steam bath for a short time, when the colour of the solution changed to yellowish-brown. The sodium salt of o-nitrophenylthioglycollic acid was now in solution, from which the free acid was precipitated on addition of dilute sulphuric acid till acid to congo red paper. The acid was filtered off and for purification it was found preferable to redissolve in sodium carbonate solution, filter and reprecipitate with dilute sulphuric acid, rather than recrystallise from aqueous alcohol from which it forms fine yellowish-brown matted needles melting-point 164°. Claasz gives melting-point 163-164°.

Yield: 26g. (47% theoretical)

3 - Keto - 2:3 - dihydrobenz - 1:4 - thiazine

3 - Keto - 2:3 - dihydrobenz - 1:4 - thiazine was prepared by a modification of the method of Claasz (loc. cit. 751). o-Nitrophenylthioglycollic acid (10g) was thoroughly mixed with a quantity of zinc dust, and glacial acetic acid (40 c.c.) added quickly. The flask was immediately fitted with an air condenser as a vigorous reaction took place a
few seconds after the addition of the acid. When
the reaction had abated the flask was warmed over a
small flame and the reduction allowed to proceed
until the liquid was pale amber in colour. Small
quantities of fresh zinc dust were added at intervals
during the reduction. The product was then filter-
ed from the zinc and the residue washed three times
with small quantities of hot glacial acetic acid
and the washings added to the filtrate. The solu-
tion in glacial acetic acid was poured into excess
cold water, when a cream precipitate was obtained,
which was filtered off, dried and had a melting-
point of 170-172°. The impure compound was dis-
solved in boiling alcohol and the solution poured
into an excess of cold water. The precipitate was
filtered off, dried and had a melting-point of 176°.
Claasz gives melting-point 176°, and Rogers and
Sexton (J.C.S. 1947, 1621) who obtained the benz-
thiazine derivative by another method gives melting-
point 179°.

Yield: - 6.0g. (74% theoretical).
6-AMINO-3-KETO-2:3-DIHYDROBENZ-1:4-
THIAZINE

The amino compound was prepared according to the following series of reactions

2:4 - Dinitrophenylthioglycollic acid

2:4 - Dinitrophenylthioglycollic acid was prepared by the method of Friedlaender and Chwala (Monatsh., 1907, 28, 276).

Thioglycollic acid (40g), 2:4 - dinitrochlorobenzene (80g) and fused sodium acetate (200g) were dissolved in alcohol (1 litre) and heated to boiling. After a few minutes the reaction mixture formed a paste of yellow crystals, which consisted of the sodium salt of dinitrophenylthioglycollic acid. The crystals were filtered off, washed with alcohol (200 c.c.) and then dissolved in water (2 litres). The free acid was obtained from this solution of the sodium salt on addition of dilute sulphuric acid and was found to be pure enough for the next stage in the synthesis. It had a melting-point of 166-172°. On crystallisation from alcohol yellow needles melting-point 172° were obtained. Friedlaender and Chwala give melting-point 167-168°. The preparation was repeated several times, 950g.
thioglycollic acid giving 2023 g. 2:4 - dinitrophenylthioglycollic acid, representing a yield of 84% of the theoretical.

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine

2:4 - Dinitrophenylthioglycollic acid was converted to 6 - amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine by reduction with tin and hydrochloric acid. The dinitro acid (40g) was mixed with granulated tin (100g) in a 1000 c.c. round-bottomed flask and concentrated hydrochloric acid (320 c.c.) added. The flask was then warmed carefully on a steam bath till the colour of the nitrocompound had gone. The reduction may become very vigorous if the heating is not carried out carefully.

To the reaction mixture was added concentrated hydrochloric acid (60 c.c.) and the mixture allowed to cool when white needles of the tin double salt separated. These were filtered off, dissolved in water (600 c.c.) and the solution filtered to remove any impurities and excess tin. Hydrogen sulphide was then passed into the filtrate until all the tin was precipitated. The tin sulphide was filtered off and washed well with hot water until the washings gave no precipitate on making alkaline with sodium hydroxide solution. The filtrate and washings were then made alkaline with sodium hydroxide solution, when white needles were precipitated. The crystals
were filtered off and recrystallised from water (2800 c.c.) as colourless needles melting-point 222-224°. The preparation was repeated several times, 2023g. 2:4 - dinitrophenylthioglycollic acid giving 910g. 6 - amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine representing a yield of 65% of the theoretical.

Friedlaender and Chwala (loc. cit. 277) give melting-point 222-224° but they give very little detail for this reduction and preferred using iron filings and acetic acid at 100°. It was found that this method was unsatisfactory. Reduction in alcoholic solution with hydrochloric acid and iron filings (West's Method J.C.S. 1925, 127, 494) also proved unsatisfactory.

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine, hydrochloride

The amino compound (6g) was dissolved in hot dilute hydrochloric acid (225 c.c.) and concentrated hydrochloric acid (75 c.c.) added. On cooling white needles of the hydrochloride were obtained. The hydrochloride was filtered off and dried in a vacuum desiccator. The crystals did not melt but decomposed at 272-274°. Friedlaender and Chwala give few details but give melting-point 272-274°.

Yield: 6.0g. (83% theoretical).

6 - Acetamino - 3 - keto - 2:3 - 3 dihydrobenz - 1:4 - thiazine

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 -
thiazine (5g) was refluxed with excess acetic anhydride over a period of 16 hours. The solution was cooled, poured into an excess of cold water, filtered, the filtrate evaporated to small bulk and allowed to cool. The crystals formed on cooling were filtered off, recrystallised from glacial acetic acid, washed and dried. The crystals had a melting-point 257°. Friedlaender and Chwala (loc. cit.) give melting-point 257° but few details of the preparation.

Yield: 4.0g, (65% theoretical)

6-Fluoro-3-keto-2:3-dihydrobenz-1:4-thiazine

6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine (4.5g), with concentrated hydrochloric acid (10 c.c.) and water (100 c.c.), was diazotised below 10° with 2N sodium nitrite solution (20 c.c.) and any excess nitrite destroyed by adding a little urea.

To the cold diazonium solution was added, with stirring, 40% fluoboric acid solution (20 c.c.) and the mixture stirred for two hours. A pale yellow precipitate of the diazonium fluoborate separated. This was filtered off, washed with water, alcohol and ether and dried. Yield 5.5g.

The diazonium fluoborate was then decomposed by heating in the vapour from boiling xylene (135° approximately), the heating being continued for 30 minutes after decomposition was apparently completed. The decomposition was carried out in the apparatus shown in Figure 1. The product after
FIGURE 1

Apparatus used in preparation of
6-Fluoro-3-Keto-2:3-Dihydrobenz-1:4-Thiazine

Diagram:
- Condenser
- Special Flask (Bulb capacity 60cc)
- 1½ Litre Bolt Neck Flask
- Diazonium Fluoborate
- Xylene
- In
- Out
- 1½ Litre Bolt Neck Flask
decomposition was then extracted for 30 minutes with boiling absolute alcohol, filtered hot and allowed to cool. An orange-brown precipitate was obtained, this impure material was filtered off and discarded. The filtrate was warmed to boiling, diluted with water until a precipitate was just formed, reheated to boiling and allowed to cool. The impure fluoro-compound crystallised out in pale yellow crystals. This was filtered off, dried and found to have a melting-point of 178-180°. The impure material was recrystallised from aqueous alcohol until the melting-point was constant.

Final product:-

pale yellow platelets melting-point 184°

(Found: C, 52.44; H, 3.17; N, 7.70.

C₈H₆OFNS requires C, 52.45; H, 3.28; N, 7.65 %).

The preparation was repeated 28g. of the 6-amino compound giving 7.5g. of the fluoro-compound representing a yield of 26% of the theoretical.

6-Chloro - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine

6-Chloro - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine was prepared according to the method outlined by Friedlaender and Chwala (loc. cit. 278). 6-Amino - 3 keto - 2:3 - dihydrobenz - 1:4 - thiazine (4.5g) was diazotised as in the previous preparation.

The diazonium solution was then added to cold
10% cuprous chloride solution (25 c.c.), much frothing occurred and the mixture was stirred until the reaction had ceased. The product was then refluxed with alcohol (100 c.c.) for 30 minutes, filtered from the cuprous chloride and the filtrate boiled with animal charcoal for at least one hour. This was filtered and the 6-chloro compound crystallised in colourless needles from the filtrate on cooling. It had a melting-point of 204°, Friedländer and Chwala gives melting-point 205°.

Yield: - 3.0g (60% theoretical).

Cuprous Chloride Solution

Crystallised copper sulphate (100g), sodium chloride (48g) and water (200 c.c.) were heated to boiling, concentrated hydrochloric acid (400g) and copper turnings (72g) were added and the whole gently boiled until decolourised. It is important to exclude air from the flask therefore a glass wool plug was used. The solution was then rapidly decanted from the unchanged copper and concentrated hydrochloric acid added until the total weight was 815g. This gave about 10% cuprous chloride solution.

* 6-Bromo-3-keto-2:3-dihydrobenz-1:4-thiazine

Although several attempts were made, using two different methods of preparation, this compound could not be isolated in an analytically pure state.
The methods of preparation used were as follows:

(a) By the Sandmeyer reaction.

6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine was diazotised below 10°C using sulphuric acid and the diazonium solution added gradually, with rapid stirring, to cold freshly prepared cuprous bromide solution. The mixture was stirred for 6 hours after which alcohol was added and the whole gently refluxed on the water bath for one hour. This was then filtered hot and the crude bromo-compound crystallised from the aqueous alcoholic filtrate on cooling. This material was recrystallised from aqueous alcohol, until of constant melting-point.

The analyses and melting-points of the products are given below.

<table>
<thead>
<tr>
<th>% Nitrogen</th>
<th>Melting-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.84</td>
<td>157-158°</td>
</tr>
<tr>
<td>8.64</td>
<td>158-160°</td>
</tr>
<tr>
<td>9.14</td>
<td>161°</td>
</tr>
<tr>
<td>8.30</td>
<td>165°</td>
</tr>
</tbody>
</table>

C₈H₆OBrNS requires N: 5.74%

(b) By perbromide

The 6-amino compound was diazotised as above and the diazonium solution added dropwise, with stirring, to an ice cold solution of bromine in potassium bromide. When the addition was complete the temperature was allowed to rise gradually to room temperature, the mixture being constantly
stirred. The diazonium perbromide which separated was filtered off, washed with cold water and dried in a vacuum desiccator.

The perbromide was then decomposed by heating in the vapour from boiling bromobenzene (154° approximately) for varying periods of time, the product extracted with boiling absolute alcohol for one hour, filtered hot and the crude bromo-compound crystallised from the filtrate on cooling. This crude material was recrystallised from aqueous alcohol until of constant melting-point. The following results were obtained.

<table>
<thead>
<tr>
<th>Time of heating</th>
<th>% Nitrogen</th>
<th>Melting-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>6.72</td>
<td>208-210°</td>
</tr>
<tr>
<td>8 hours</td>
<td>4.97</td>
<td>220°</td>
</tr>
<tr>
<td>12 hours</td>
<td>4.58</td>
<td>220-224° (decomposition)</td>
</tr>
</tbody>
</table>

The products obtained by both methods all contained bromine, the nearest approach to the pure compound being the product melting at 220° and containing 4.97% as against 5.74% N.

6 - Iodo - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine
6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine (4.5g) was diazotised below 10° using concentrated sulphuric acid (4 c.c.), water (100 c.c.) and 2N sodium nitrite solution (20 c.c.). To the diazonium solution was added, with rapid stirring, a solution of potassium iodide (8g)
in the minimum of water and the whole stirred for 5 hours. Alcohol (200 c.c.) was then added and the alcoholic solution gently heated to boiling on a water bath. The solution was refluxed for one hour, filtered hot and the crude iodo-compound crystallised from the aqueous alcoholic filtrate. This crude material, melting-point 203°, was recrystallised from aqueous alcohol until of constant melting-point.

Final product:

Feathery orange needles, melting-point 208 - 210°

Yield: 3.5g. (48% theoretical)

(Found: I, 43.52; N, 5.08; C₈H₆OINS
requires I, 43.62; N, 4.81%)

6-Thiocyanato-3-keto-2:3-dihydropyrrrolo[1:4]thiazine

6-Amino-3-keto-2:3-dihydropyrrrolo[1:4]thiazine (4.5g) was diazotised as in the above preparation.

To the diazonium solution was added, with rapid stirring, a solution of potassium thiocyanate (4g) in water followed by a paste prepared in the following way. To a solution containing crystalline copper sulphate (8g) and ferrous sulphate (16g) was added a solution of potassium thiocyanate (4g), this was then filtered at the pump and the residue on the filter made into a paste with water. The mixture
was rapidly stirred for 9 hours. Alcohol (200 c.c.) was then added, the mixture gently warmed to boiling on a water bath, refluxed for 1.5 hours, filtered hot and the aqueous alcoholic filtrate allowed to cool. Orange crystals melting-point 159° separated. These were then recrystallised from aqueous alcohol until of constant melting-point.

Final product:-

pale yellow needles, melting-point 180°

Yield:- 3.0g. (54% theoretical)

(Found:  C, 48.43;  H, 3.06;  N, 12.30;
C₉H₆O₂N₂S₂ requires  C, 48.62;
H, 2.71;  N, 12.60%)

6-Triazo-3-keto-2:3-dihydrobenz-1:4-thiazine

6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine (4.5g) was diazotised in the usual way using sulphuric acid.

The diazonium solution was cooled in ice and a solution of sodium azide (2g) slowly added with stirring, nitrogen was evolved and a white precipitate formed. The mixture was kept cool in ice and stirred for 5 hours. The temperature was then allowed to rise to room temperature, the precipitate filtered off, washed with water and dried. This product was recrystallised from aqueous alcohol until a constant melting-point was obtained.
Final product:

Pale yellow feathery needles turning yellowish brown with slight decomposition on exposure to light, melting-point 176° (decomposition).

Yield: 5.0g. (97% theoretical)
(Found: N, 26.98; C₈H₆O₄N₄S requires N, 27.18%)

6-Nitro-3-keto-2:3-dihydrobenz-1:4-thiazine

This compound was prepared by a method similar to that of Hodgson and Marsden (J.C.S. 1944, 22).

6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine (4.5g) was diazotised in the usual way using hydrochloric acid. The diazonium solution formed was neutralised with calcium carbonate and filtered.

Finely powdered sodium cobaltinitrite (11g) was then added to the filtrate with good stirring and the aryl diazonium cobaltinitrite, which rapidly separated, filtered off and air dried.

The finely powdered diazonium cobaltinitrite (10g) was added in portions, at room temperature, to a well stirred solution of sodium nitrite (10g) and crystallised copper sulphate (10g) in water (60 c.c.) in which cuprous oxide (4g) was suspended. The mixture was stirred until evolution of nitrogen had ceased, filtered and the filtrate discarded.

The greenish residue on the filter was refluxed with alcohol, filtered hot and the crude nitro-
compound crystallised from the alcoholic filtrate. The crude compound was purified by recrystallising from aqueous alcohol. The experiment was repeated several times.

Final product:

golden brown feathery needles, melting-point 243-244\degree C.

Total yield: 14g.6 - amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine gave 3.0g. 6 - nitro - compound (19% theoretical).

(Found: N,13.62; C₈H₆O₃N₂S requires N, 13.33%).

6 - Nitroso - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine

This compound was prepared by a method similar to that described by Bamberger and Landsteiner (Ber. dtsch. chem. Ges., 1893, 26, 482).

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine (4.5g) was diazotised below 10\degree C using concentrated hydrochloric acid (10 c.c.), water (100 c.c.) and 2N sodium nitrite solution (20 c.c.). Any excess nitrite was destroyed by adding a little urea.

The diazonium solution was run into 4% potassium permanganate solution (70 c.c.) at 0\degree-10\degree C and the whole made alkaline with potassium hydroxide solution. The mixture was stirred for 3 hours after which it was extracted with ether. Any emulsion formed was broken up by adding a few drops
of a wetting agent. The ethereal layer was separated, the ether distilled off and the residue dissolved in alcohol. This solution was then filtered hot and the nitroso-compound crystallised in pale yellow platelets from the aqueous alcoholic filtrate on cooling.

The nitroso-compound was recrystallised from aqueous alcohol until the melting-point was constant. The experiment was repeated several times.

Final product:

- pale yellow platelets melting-point 134°.
- Total yield: 22.5g.
- 6 - amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine gave 1.0g. 6 - nitroso-compound (4% theoretical).
- (Found: N, 14.73; C₉H₆O₂N₂S requires N, 14.43%)

3 - Keto - 2:3 - dihydrobenz - 1:4 - thiazine - 6 - thiol

This compound was prepared by a method similar to that of Leuckart (J. pr. Chem. 1890, (11) 41,179).

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine (4.5g) was diazotised in the usual way using hydrochloric acid.

To the well stirred, ice-cold, diazonium solution was added an ice-cold solution of potassium xanthate, C₂H₅O-CS-SK (4g) in water. The mixture was cooled in ice and vigorously stirred for 2 hours, when a bright yellow precipitate was obtained. The ice was then removed and the
temperature of the mixture allowed to rise gradually to room temperature. The decomposition was completed by heating on the water bath for 1.5 hours.

The yellow precipitate formed was filtered off, dried and weighed (23g). This was then dissolved in alcohol, potassium hydroxide (17g) added and the mixture refluxed until a sample remained clear on the addition of water (approximately 1 hour). The alcohol was evaporated off, water added, the solution filtered and the filtrate acidified with dilute sulphuric acid. The creamy yellow precipitate which resulted was filtered off and dried. This was then recrystallised from aqueous alcohol until a constant melting-point was obtained.

Final product:–

pale yellow needles melting-point 174°.

Yield:– 3.0g. (61% theoretical)

(Found:– C, 48.94; H, 3.22; N, 7.39; 
C₈H₇O₂NS₂ requires C, 48.73; H, 3.55; 
N, 7.11%)

3-Keto-2:3-dihydrobenz-1:4-thiazine-6-arsonic acid

This compound was prepared by a method similar to that of Bart (Annalen, 1922, 429, 55).

6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine (4.5g) was diazotised in the usual way using hydrochloric acid.

To the diazonium solution, cooled in ice, was added with rapid stirring, a solution of sodium
arsenite Na$_3$AsO$_3$ (5g) in water (25 c.c.), and whilst the mixture was stirring 10% aqueous sodium hydroxide was added until the mixture was alkaline to litmus. The mixture was then stirred until it gave no further test for a diazo-compound (3-4 hours). The solution was filtered cold through a fluted filter paper, the filtrate made slightly acid to congo red and boiled with granular animal charcoal for 1 hour. This was filtered hot, the filtrate on testing was found to be alkaline to litmus and was evaporated to small bulk, made strongly acid with concentrated hydrochloric acid and further evaporated until crystals were formed.

Brownish coloured crystals were obtained on cooling. The crystals were sparingly soluble in cold water but readily soluble in hot water and on testing were found to contain arsenic, sulphur and nitrogen.

The crude arsonic acid was purified as follows. The crystals were dissolved in hot water, cooled and acetone added until precipitation was complete. The precipitate formed was filtered off and found to contain arsenic but no sulphur or nitrogen. The filtrate was evaporated until crystallisation commenced and allowed to cool. This gave brownish coloured crystals which were tested and found to contain arsenic, sulphur and nitrogen. This product was then further purified by recrystallisation from hot
water. The experiment was repeated several times.

Final product:

pale yellow plates, do not melt but decompose gradually above 300°C.

Total yield: 27g. 6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine gave 3.0g. arsanic acid (7% theoretical).

(Found: C, 33.28; H, 2.67; N, 5.03; C₈H₈O₄N₂S₂As requires C, 33.22; H, 2.77; N, 4.34%)

3-Keto-2:3-dihydrobenz-1:4-thiazine-6-stibonic acid

6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine (4.5g) was diazotised in the usual way using hydrochloric acid.

To the diazonium solution, cooled in ice, was added with rapid stirring, a solution of antimony trioxide, Sb₂O₅, (8g) in concentrated hydrochloric acid. Whilst the mixture was being stirred, 10% aqueous sodium hydroxide was added until an alkaline reaction was obtained. The stirring was then continued until the mixture gave no further test for a diazo-compound. The mixture was filtered, the filtrate made slightly acid to congo red with dilute hydrochloric acid, boiled for 1 hour with animal charcoal, made alkaline and filtered hot. The filtrate was then made strongly acid with concentrated hydrochloric acid and the purple-pink
precipitate which resulted filtered off. The filtrate was discarded.

The precipitate was dissolved in hot sodium carbonate solution, filtered hot, the filtrate acidified with dilute sulphuric acid and allowed to cool. This gave a reddish-brown precipitate which was further purified by redissolving in sodium carbonate solution and reprecipitating with dilute sulphuric acid. The preparation was repeated several times.

Final product:

reddish-brown amorphous powder with no definite melting-point but decomposing gradually above 270°.

Total yield: - 13.5g. 6 - amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine gave 3.5g. stibonic acid (14% theoretical)

(Found: C, 28.84; H, 2.50; N, 3.88; C₈H₈O₄N S Sb requires C, 28.59; H, 2.38; N, 4.17%)

3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine - 6 - mercuric chloride

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine (4.5g.) was diazotised in the usual way using hydrochloric acid.

The diazonium solution was added, with rapid stirring, to a solution of mercuric chloride (7g.) in concentrated hydrochloric acid (7 c.c.) mixed
with ice (7g). The mixture was cooled in ice and stirred for 2 hours. The diazonium mercuric chloride which separated was filtered off and dried in a vacuum desiccator. Yield 19g. (approximately).

This compound was then mixed with acetone (80 c.c.) cooled in ice and treated with copper bronze (6g). The mixture was stirred for 3 hours, filtered and the filtrate discarded. The residue was heated to boiling with nitrobenzene, filtered hot and the mercuric chloride compound crystallised from the filtrate in pale yellow needles on cooling.

The preparation was repeated several times, 27g. of the 6 - amino compound giving 3.0g. of the mercuric chloride representing a yield of 5% of the theoretical.

Final product:

- pale yellow needles, melting-point 263-264° (decomposition).

(Found: C, 24.31; H, 1.24; N, 3.40;
C₈H₆O Cl N S Hg requires C, 24.00; H, 1.50;
N, 3.50%)

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine → phloroglucinol

6 - amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine (4.5g) was diazotised in the usual way using hydrochloric acid.

The diazonium solution was added rapidly, with stirring, at a temperature below 10° to a solution of phloroglucinol (3.5g) in 2N sodium hydroxide.
solution (38 c.c.) and 2N sodium carbonate solution (150 c.c.). A dark reddish-brown precipitate resulted and the mixture stirred for 6 hours. The precipitate was filtered off, washed with cold absolute alcohol (50 c.c.) in 10 c.c. portions and finally with acetone (10 c.c.). It was then removed from the filter, dried in a vacuum desiccator and finally in a low oven.

Final product:-

deep purple coloured amorphous powder with no melting-point but decomposing above 300°.

Yield:- 9g. (94% theoretical).

(Found: N, 10.93; \( \text{C}_{14}\text{H}_8\text{O}_4\text{N}_3\text{S} \text{Na}_3 \) requires N, 10.97%)

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine \( \longrightarrow \) salicylic acid

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine (4.5g) was diazotised in the usual way using hydrochloric acid.

The diazonium solution was added rapidly, with stirring, at a temperature below 10° to a solution of salicylic acid (3.5g) in 2N sodium hydroxide solution (25 c.c.) and 2N sodium carbonate solution (100 c.c.). A yellowish-brown precipitate resulted and the mixture stirred for 6 hours. The precipitate was filtered off, washed with cold absolute alcohol (50 c.c.) in 10 c.c. portions and finally with acetone (10 c.c.). It was then removed from the filter, dried in a vacuum desiccator and finally in
a low oven.

Final product:-

golden brown coloured amorphous powder with no melting-point but decomposing at 280°.
Yield:-- 9g. (97% theoretical).
(Found: N, 10.81; C_{15}H_{9}O_{4}N_{3}S Na requires N, 11.26%)

6:6' - bis - (3 - Keto - 2:3 - dihydrobenz - 1:4 - thiazine)

This compound was prepared according to the method of Gattermann and Ehrhardt (Ber. dtsch. chem. Ges. 1890, 22, 1226) for the preparation of diphenyl.

6 - Amino - 3 - keto - 2:3 - dihydrobenz - 1:4 - thiazine (4.5g) was diazotised in the usual way using sulphuric acid.

To the diazonium solution was added 95% alcohol (12 c.c.) followed by copper bronze (5g), which was introduced slowly, the mixture being stirred during addition and for 1 hour afterwards keeping the temperature below 30°. The temperature was then raised slowly on the water bath to a maximum of 75°, when a vigorous reaction took place and a reddish-brown product was formed. This was filtered off and the filtrate discarded.

The residue was warmed on the water bath with dilute nitric acid in order to remove any excess copper, filtered and thoroughly washed with water until free from nitric acid. The brown product
on the filter was then refluxed on the water bath with absolute alcohol, filtered hot and dried in a vacuum desiccator. The filtrate contained impurities and was discarded.

Final product:—

brown amorphous powder, melting-point above 330°.

Yield: 3.5g. (43% theoretical).

(Found: N, 8.31; C₁₆H₁₂O₂N₂S₂ requires N, 8.54%)
3 - KETO - 2:3 - DIHYDRO - 6:7 - DIMETHOXYBENZ - 1:4 - THIAZINE

3 - Keto - 2:3 - dihydro - 6:7 - dimethoxybenz - 1:4 - thiazine was prepared by the following series of reactions

Veratrole

Veratrole was prepared according to the method of Ullmann (Annalen, 1903, 327, 115). Catechol (44g) was dissolved in 20% sodium hydroxide solution
(200 c.c.) and dimethyl sulphate (80 c.c.) added to the solution, which was rapidly turning brown, and vigorously agitated. The liquid became warm and, after cooling, the veratrole formed was extracted with ether and the ethereal extract dried over anhydrous sodium sulphate. After distilling off the ether the residue was distilled and collected at 206°. A total yield of 101g. was obtained from 132g. catechol representing a yield of 61%.

**Veratrole - 4 - sulphonyl chloride**

Veratrole - 4 - sulphonyl chloride was obtained according to Brown and Robinson (J.C.S. 1917, 111, 953). Chlorosulphonic acid (160g.) was dissolved in chloroform (480g.) and the solution gradually added to veratrole (80g.). A vigorous reaction occurred and it was found more satisfactory to keep the reaction mixture well stirred during the addition and during the hour afterwards. After this interval, water was gradually added, the chloroform layer separated and dried over anhydrous sodium sulphate. After evaporation away of the chloroform, the residue was sufficiently pure for the following preparation. A small portion of the crude material was crystallised from a mixture of benzene and light petroleum when colourless needles were obtained, melting-point 69°. Brown and Robinson give 71°. A total yield of 111g. was
obtained from 101g. veratrole representing a yield of 64% theoretical.

3:4 - Dimethoxyphenylthiol

3:4 - Dimethoxyphenylthiol was obtained by the method of Fries, Koch and Stukenbrock (Annalen. 1929, 468, 172). Veratrole - 4 - sulphonyl chloride (64g.) and zinc dust (60g.) were mixed in a flask with stirring. Ether (80 c.c.) was added and the flask was fitted with a reflux condenser. Concentrated hydrochloric acid (192 c.c.) was dropped slowly down the reflux into the flask and then more quickly until about half of the hydrochloric acid had been added. It is advisable to cool the flask as the reaction tends to be rather vigorous. This took about 45 minutes. The flask was then heated gently on the water bath for 45 minutes whilst the rest of the hydrochloric acid was added. The flask was then heated to boiling and the contents maintained at boiling point for 3.5 hours, after which time the reduction to the thiol was complete, and the thiol was seen floating on the surface of the reaction mixture as a yellow oil. Ether was added, the ethereal layer washed with water to remove any zinc chloride and dried over anhydrous sodium sulphate. After removal of the ether the thiol was pure enough for the next stage in the synthesis. Repetition of the experiment gave a total yield of 77g. from 111g. veratrole - 4 - sulphonyl chloride
representing a yield of 97%.

3:4 - Dimethoxyphenylthioglycollic acid

3:4 - Dimethoxyphenylthioglycollic acid was prepared by the method of Baldick and Iyons (J. roy. Soc. N.S.W. 1937-38, 71, 113). The thiol (35g), prepared above, was added to 25% sodium hydroxide solution (600 c.c.) when the sodium salt separated as a creamy mass. This mixture was added to a solution of sodium chloro-acetate obtained by dissolving monochloroacetic acid (35g) in water (35 c.c.) and adding anhydrous sodium carbonate till a neutral solution was obtained. The mixture was heated to about 50° and then allowed to stand for 2 hours. The solid sodium salt soon passed into solution and the solution was filtered. Concentrated hydrochloric acid was added to the filtrate when the dimethoxyphenylthioglycollic acid separated in white needles melting-point 101-102°. This was sufficiently pure for nitration. A little was crystallised from aqueous alcohol as white needles, melting-point 106°. Baldick and Iyons give melting-point 106°. The experiment was repeated several times. 77g. 3:4 - dimethoxyphenylthiol giving a yield of 90g. 3:4 - dimethoxyphenylthioglycollic acid (87% theoretical).

2 - Nitro - 4:5 - dimethoxyphenylthioglycollic acid

The nitro-acid was obtained by the method of Baldick and Iyons (loc. cit. 114). 3:4 - Dimeth-
oxyphenylthioglycollic acid (60g.) was dissolved in glacial acetic acid (240 c.c.) and concentrated nitric acid (D = 1.42, 60 c.c.) was added gradually. Great heat was evolved and the temperature was maintained at 20° or lower by cooling in an ice-bath. The solution became red and then a thick yellow precipitate was thrown down. The precipitate was filtered off, washed with water, and recrystallised from aqueous alcohol when the acid was obtained as yellow matted needles, melting-point 222°. Baldick and Lyons give melting-point 222°. A total yield of 86g. (80% theoretical) was obtained from 90g.

3 - Keto - 2:3 - dihydro - 6:7 - dimethoxybenz - 1:4 - thiazine

3 - Keto - 2:3 - dihydro - 6:7 - dimethoxybenz - 1:4 - thiazine was prepared according to Baldick and Lyons (loc. cit. 116).

2 - Nitro - 4:5 - dimethoxyphenylthioglycollic acid (15g.) was mixed with granulated tin (36g.) and concentrated hydrochloric acid (120 c.c.) added. The mixture was heated on the water bath until the colour of the nitro-compound had completely disappeared. On cooling white needles separated, water (600 c.c.) was added, the precipitate filtered off and recrystallised from aqueous alcohol, when it formed white matted needles melting-point 186-187°. Baldick and Lyons give melting-point 186-187°.
The experiment was repeated several times 86g. 2 - nitro - 4:5 - dimethoxyphenylthioglycollic acid giving 52g. of the dimethoxy-compound representing a yield of 75%.

3 - Keto - 2:3 - dihydro - 6:7 - dihydroxybenz - 1:4 - thiazine

3 - Keto - 2:3 - dihydro - 6:7 - dimethoxybenz - 1:4 - thiazine (5g) was boiled gently under reflux with a large excess (100 c.c.) of constant boiling hydriodic acid (b.p. 126-127°) for 2 hours. The methyl iodide formed during the demethylation was distilled off on the water bath and the residue diluted with a large volume of cold water when brownish coloured crystals separated. The crystals were filtered off at the pump, washed with cold water and recrystallised from water in the following manner.

The crystals were refluxed with boiling distilled water, filtered hot and the filtrate allowed to cool in a tightly stoppered flask. It was found necessary to exclude air as much as possible during the crystallisation as the crystals developed a deep pink colour on exposure to air.

Final product:

pale pink, almost colourless, rectangular plates decomposing above 240°. Aqueous sodium hydroxide gives a purple colour changing quickly to sky blue, but there is no
colcuration with ferric chloride.
The experiment was repeated several times; 20g. of the dimethoxy-compound giving 4g. of the dihydroxy, representing a yield of 23%.

(Found: C, 48.76; H, 3.24; N, 7.00; C₈H₇NO₂S requires C, 48.73; H, 3.55; N, 7.14%)
**EXPERIMENTAL**

**In-vitro testing of 3-keto-2:3-dihydrobenz-1:4-thiazine and derivatives.**

The method of in-vitro testing used was, with the exception of a few minor modifications, that evolved by Baldwin (Parasitol. 1943, 35, 89).

**Collection of the material**

The animals used in the tests were collected at the abattoir, Slateford, Edinburgh and placed in Ringer's solution at 38-40° contained in a vacuum flask. On reaching the Heriot-Watt College the worms were immediately transferred to fresh Ringer's solution at 38°, any worm that appeared to be in poor condition being rejected. The worms were then maintained at 38° in a thermostatically controlled bath.

As both liver fluke and Ascaris were used, two types of Ringer's solution were necessary, but it was found that the Ascarids could be kept in good condition until they reached the laboratory by placing them in the Ringer's solution used for liver fluke. This fact proved most convenient as it was not always possible to have the Ringer's solution corresponding to the material available at the abattoir, therefore for transportation liver
fluke Ringer's solution was used in both cases. The Ascarids on arrival in the laboratory were immediately transferred to Ascaris Ringer's solution at 38°.

Apparatus

A general lay-out of the apparatus used for in vitro testing is shown in figure 2. The main part of the assembly was contained in a glass tank (A) containing water which was stirred by a small electric motor (E) and electrically maintained at 37–38°.

The Ringer's solution employed was stored in a 10-litre aspirator (C) and gravity fed to two glass spirals (D) where it acquired the temperature of the bath. From the spirals the Ringer's solution passed to the test chamber (E) which it entered at the bottom, the overflow passing out at the top.

The test chamber, a full-scale drawing of which is shown in figure 3, was fitted with a tap (F) by means of which the flow of medium could be stopped if required. By turning the three-way tap (G) to the correct position the chamber could be emptied from the bottom.

No provision for oxygenation of the Ringer's solution was made as oxygenation was found to make no difference to the behaviour of the helminth preparation.

The lower end of the preparation was attached to the glass hook (H) and the upper end to the recording lever (I, fig.2) which gave a magnifica-
The movement of the lever was recorded on a smoked drum (J, fig. 2) which revolved at approximately 6 m.m. per minute, the minute intervals being recorded by a glass writing-point (K, fig. 2), operated by a clockwork timing device which incorporated a 24-volt relay (L, fig. 2).

Preparation of the compounds for testing.

The compounds which were soluble in the Ringer's solution were tested by dissolving the desired weight in the requisite volume of solution to give the concentration required.

The insoluble compounds were either tested as emulsions or as fine suspensions. Suspensions were only used when it was found impossible to form a stable emulsion.

The emulsions were prepared by dissolving the solid in absolute alcohol and adding the solution, with violent shaking, to hot Ringer's solution containing 0.05% sodium glycocholate. Preparations of this kind should not contain more than 0.5% alcohol. Liverfluke or Ascaris Ringer's solution was used to prepare the emulsion when the corresponding material was being used for testing. The pH of each solution, emulsion or suspension was determined approximately using B.D.H. pH papers, the limits being pH 7.0 to 8.6 for liverfluke and 6.0 to 8.0 for Ascaris.
Preparation of Ringer's Solution (Ascaris)

The Ringer's solution used took the form of a balanced saline medium and was kept in the form of a concentrated stock of the following composition.

- Sodium chloride 80g/litre
- Potassium chloride 2g/litre
- Calcium chloride (anhydrous) 2g/litre
- Magnesium sulphate, $7H_2O$ 1g/litre

For use, one volume of this stock solution received one tenth of its volume of 0.05M phosphate buffer at pH 6.4 and was made up to ten volumes with distilled water. The product is referred to in what follows as 'Ascaris Ringer'.

Baldwin gives no indication of the nature of the phosphate buffer used and in preparing the 'Ascaris Ringer' for use in this investigation a potassium dihydrogen phosphate / borax buffer at pH 6.4 was used.

Method of Test employed with Ascaris

The preparations of Ascaris used in the in vitro tests were prepared in the following manner.

Female worms only were used and those of rather more than average size were preferred. Only those showing a good rosy colour and considerable activity were used and, in general, no worm was used that had been in the laboratory for more than 48 hours. The worms were kept in good condition by changing the 'Ascaris Ringer' at intervals of 1.5 to 2 hours.

A suitable worm was selected, held horizontally
and dropped from a height of about 12 inches on to the bench. This caused the whole body to contract and the worm became, and remained, almost motionless long enough to allow the necessary operations to be performed. When this preliminary treatment was omitted it was found that the preparation had a tendency to contract when the ligatures were applied and became too short to give satisfactory tracings.

The 'intermediate' preparation was made by tying a ligature with fine silk thread at a position 2.8 cm. in front of the genital pore or waist. A second ligature was then applied about 3 m.m. in front of the pore and the remainder of the worm cut away. This operation was most conveniently carried out on a glass plate which had lines etched at distances of 2.8 cm. and 3 m.m. apart.

The 'anterior' preparation was made by tying a ligature in or immediately behind the groove which lies at the base of the lips i.e. slightly less than 0.5 m.m. behind the anterior extremity of the worm. A second ligature was tied 2 cm. further back and the rest of the worm cut away.

If the fragments were prepared in the above manner and the ligatures not tied tightly enough to cut into the tissues, the majority of the preparations settled down to give tracings with good rhythmic movement and a steady base line. The normal practice was to set up three or four preparations at one time keeping each in a separate boiling-
tube containing 'Ascaris Ringer' at 38°. A test-load of 0.5 g. was attached to each preparation and it was then possible to select the most suitable ones for testing. In general, preparations which showed no signs of recovery within one hour were discarded.

The preparation selected for testing was transferred from the boiling-tube to the test-chamber, the lower extremity attached to the glass hook and the upper attached to the lever. The lever was then weighted with plasticine to give a tracing of suitable amplitude, the average weight required being 1.0 to 1.5g. 'Ascaris Ringer' was passed through the test chamber at approximately 30 c.c. per minute and when the preparation had recovered its normal rhythmic movement, usually in about five minutes, the drum was set in motion and tracing commenced. The normal movement of the preparation was recorded for 15 minutes, after which the flow of 'Ascaris Ringer' was stopped, and the 'Ascaris Ringer' in the test-chamber syphoned off. The solution, emulsion or suspension, preheated to 38°, of the compound to be tested was then added, by means of a filter funnel, through the outlet tube to the test-chamber with the three-way tap in such a position that the compound entered at the bottom of the chamber.

During this operation the lever and the time marker were lifted from the drum and the drum
stopped. The lever was lightly clamped in order to prevent the preparation sagging and becoming detached from the glass hook. By taking these precautions it was possible to avoid the tracings becoming marred while the chamber was being emptied and refilled.

When the compound had been introduced, the drum was set in motion and tracing restarted. The movements of the worm were then recorded for a further 30 minutes after which, if there was no change in the rhythmic movement, tracing was suspended and the worm preparation destroyed. If, however, the rhythmic movement was in any way altered the test preparation of the compound was syphoned off and 'Ascaris Ringer' allowed to pass through the test chamber at 30 c.c. per minute for 15 minutes. Restoration of normal rhythmic movement within this period indicated that the effect of the compound on the worm preparation was only temporary.

The smoked paper after removal from the drum, was varnished with a clear alcohol varnish and allowed to dry, so making the tracing permanent.

The Ascarids used in the tests were disposed of by placing in 50% aqueous potassium hydroxide. This solution, when worked up, provided a good source of glycogen.

Preparation of Ringer's Solution (Liverfluke).

This Ringer's solution which will be referred to
as 'liver-fluke Ringer' was prepared from the following stock solutions:

- Sodium chloride 90g/litre
- Sodium bicarbonate 50g/litre
- Calcium chloride (anhydrous) 24g/litre
- Potassium chloride 42g/litre

When required for use, distilled water (ca. 800 c.c.) was added to the sodium chloride solution (100 c.c.) followed by 10 c.c. of each of the other three stock solutions, the mixture being stirred. After making up to 1 litre with distilled water, 1g. glucose was added and the whole shaken thoroughly. (Private Communication from Dr. Chance).

Method of Test employed with Liver-fluke.

The liver-fluke preparations were made by the method of Chance and Mansour (Brit. J. Pharmacol. 1949, 4, 7). The liver-fluke were kept in 'liver-fluke Ringer' at 37-38° in separate boiling-tubes placed in the thermostatically controlled bath. Chance and Mansour found this necessary in order to prevent the flukes from attacking each other, which they were apt to do, when large numbers were enclosed together. The 'liver-fluke Ringer' was changed at intervals of 2 hours and by so doing the flukes were found to remain quite lively for periods up to 8 hours after removal from the host.

The liver-fluke was attached to the lever and the glass hook by means of platinum hooks made from fine platinum wire (0.4 m.m. diameter). The hooks
passed through the body wall posterior to the ventral sucker and close to the posterior end of the body. It was found essential to keep the fluke below the surface of the 'liver|fluke Ringer' whilst these attachments were being made in the shortest possible time. This operation was best performed in a Petri dish containing 'liver|fluke Ringer' at 37°. The fluke was then allowed 10 minutes to recover before the load on the lever was adjusted and recording started. The load found most suitable for liver|fluke varied from 1 to 2g.

'Liver|fluke Ringer' was passed through the test-chamber at approximately 30 c.c. per minute and the normal movements recorded for 15 minutes. The chamber was then emptied and refilled with the test preparation of the compound in the same way as for Ascaris and recording continued for 45 minutes. At the end of this period the preparation of the compound was syphoned off and amphetamine sulphate (1 in 5000) in 'liver|fluke Ringer' added and the movements recorded for a further 15 minutes. The smoked paper was then removed from the drum, varnished, and allowed to dry.

Interpretation of the Tracings.

The tracings were carried out in duplicate at each concentration of the compound, which was tested against both Ascaris and liver|fluke.

The compounds were classified as stimulant, depressant, paralysant or lethal. A compound was
said to have a stimulant effect when one or more of the following conditions were observed on the tracing.

(a) The base line was raised.
   i.e. the tone of the preparation was increased.

(b) The frequency was increased.

(c) The amplitude was increased.

A compound was classified as having no effect when no change in the normal rhythmic movement was observed.

A depressant effect was indicated by at least one of the following conditions:

(a) The base line was lowered.
   i.e. the tone of the preparation was lowered.

(b) The frequency was decreased.

(c) The amplitude was decreased.

A compound was said to be paralysant when on addition, the preparation ceased to show any signs of movement but recovered when the compound was withdrawn and amphetamine sulphate or fresh 'Ascaris Ringer' added, depending on whether liver fluke or Ascaris was being used.

If on addition of amphetamine sulphate or fresh 'Ascaris Ringer' to the preparation no recovery took place the compound was said to be lethal in its action.
RESULTS

Owing to the large number of tracings carried out it is not possible to include a photograph of each tracing. In order, therefore, to give an indication of the nature of the tracings obtained several of the most characteristic are included and the interpretation of these is described in detail.

The general results for all the compounds tested are given in tabular form immediately after the detailed accounts of the tracings.

Results obtained with Ascaris

**Tracing No. 1**

Compound: - 3-Keto-2:3-dihydrobenz-1:4-thiazine.
Concentration: - 1 in 4000 emulsion.

pH: - 6.0

Compound added after: - 11 minutes.

General action: - slight increase in amplitude.
Conclusion: - slight stimulating effect.

**Tracing No. 2**

Compound: - 3-Keto-2:3 - dihydrobenz-1:4-thiazine
Concentration: - 1 in 1000 emulsion.

pH: - 5.8

Compound added after: - 13 minutes

General action: - amplitude and frequency increased 3 minutes after addition of the compound. Frequency still further increased 17 minutes
after addition of the compound.
Conclusion:—stimulant effect.

**Tracing No. 3**

Compound:—6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine.
Concentration:—1 in 1000 emulsion.

pH:—5.9

Compound added after:—12 minutes.
General action:—very slight decrease in amplitude
Conclusion:—practically no effect.

**Tracing No. 4**

Compound:—6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine, hydrochloride
Concentration:—1 in 4000 solution.

pH:—5.8

Compound added after:—16 minutes.
General Action:—slight fall in tone, amplitude and frequency unaltered.
Conclusion:—practically no effect.

**Tracing No. 5**

Compound:—6-Acetamino-3-keto-2:3-dihydrobenz-1:4-thiazine.
Concentration:—1 in 4000 solution

pH:—6.6

Compound added after:—13 minutes
General action:—very slight fall in tone, amplitude and frequency unaltered.
Conclusion:—practically no effect.

**Tracing No. 6**

Compound:—6-Chloro-3-keto-2:3-dihydrobenz-1:4-thiazine.
Tracing No. 6 (Cont'd)

Concentration: 1 in 4000 emulsion
pH: 5.8
Compound added after: 12 minutes.
General action: no change.
Conclusion: no effect.

Tracing No. 7

Compound: 6-Thiocyano-3-keto-2:3 - dihydrobenz - 1:4 - thiazine.
Concentration: 1 in 4000 emulsion.
pH: 5.6
Compound added after: 11 minutes.
General action: very slight decrease in amplitude, no change in tone or frequency.
Conclusion: practically no effect.

Tracing No. 8

Compound: 6-Nitro-3-keto-2:3 - dihydrobenz - 1:4 thiazine.
Concentration: 1 in 1000 suspension
pH: 5.6
Compound added after: 12 minutes.
General action: no change.
Conclusion: no effect.

Tracing No. 9

Compound: 6-Nitrosso-3-keto-2:3 - dihydrobenz - 1:4 thiazine.
Concentration: 1 in 4000 emulsion
pH: 5.8
Compound added after: 15 minutes.
General action: amplitude increased after addition but frequency very slightly decreased. No change
Tracing No. 9 (Contd)

in tone.

Conclusion:-- slight stimulant effect.

Tracing No. 10

Compound:-- 3-Keto-2:3-dihydrobenz-1:4-thiazine - 6 - arsonic acid.
Concentration:-- 1 in 1000 emulsion.

pH:-- 5.6

Compound added after:-- 10 minutes.

General action:-- very slight fall in tone and amplitude decreased very slightly but frequency unaltered.

Conclusion:-- practically no effect.

Tracing No. 11

Compound:-- 3-Keto-2:3-dihydrobenz-1:4-thiazine - 6-stibonic acid.
Concentration:-- 1 in 4000 suspension.

pH:-- 5.6

Compound added after:-- 10 minutes.

General action:--, slight fall in tone, frequency and amplitude unaltered.

Conclusion:-- practically no effect.

Tracing No. 12

Compound:-- 3-Keto-2:3-dihydro-6:7-dimethoxybenz-1:4 thiazine.
Concentration:-- 1 in 2000 emulsion

pH:-- 5.8

Compound added after:-- 14 minutes.

General action:-- very slight decrease in tone. Amplitude decreased and frequency increased. All effects occur
Tracing No. 12 (Contd)

2 minutes after addition of the compound.

Conclusion: slight depressant effect.

Tracing No. 13

Compound: 3-Keto-2:3-dihydro-6:7-dimethoxybenz-1:4-thiazine.

Concentration: 1 in 1000 emulsion

pH: 5.7

Compound added after: 14 minutes.

General action: no change.

Conclusion: no effect.

Tracing No. 14

Compound: 3-Keto-2:3-dihydro-6:7-dihydroxybenz-1:4-thiazine

Concentration: 1 in 1000 solution

pH: 5.6

Compound added after: 13 minutes.

General action: no change.

Conclusion: no effect

Tracing No. 15

Compound: 3-Keto-2:3-dihydro-6:7-dihydroxybenz-1:4-thiazine

Concentration: 1 in 1000 solution plus 0.05% (by volume) "Grill No. 10"

pH: 5.8

Compound added after: 11 minutes.

General action: slight fall in tone and slight decrease in amplitude but very small increase in frequency.

Conclusion: very slight depressant effect.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Solution or Emulsion or Suspension</th>
<th>pH</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>emulsion</td>
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<td>Slight stimulant effect</td>
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<tr>
<td>6-aminoc-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
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<td>emulsion</td>
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<td>6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine, hydrochloride</td>
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<td>Solution</td>
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<td>No effect</td>
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<tr>
<td>6-acetamino-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>emulsion</td>
<td>6.6</td>
<td>No effect</td>
</tr>
<tr>
<td>6-chloro-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>emulsion</td>
<td>5.8</td>
<td>No effect</td>
</tr>
<tr>
<td>6-ido-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>5.7</td>
<td>No effect</td>
</tr>
<tr>
<td>6-thiocyano-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
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<td>emulsion</td>
<td>5.8</td>
<td>No effect</td>
</tr>
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<td>suspension</td>
<td>5.8</td>
<td>No effect</td>
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<td>emulsion</td>
<td>5.8</td>
<td>No effect</td>
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<td>6-thiophene-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
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<td>emulsion</td>
<td>5.7</td>
<td>Slight stimulant effect</td>
</tr>
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<td>9-keto-2:3-dihydrobenz-1:4-thiazine-6-thiol</td>
<td>1 in 4000</td>
<td>emulsion</td>
<td>5.6</td>
<td>No effect</td>
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<td>9-keto-2:3-dihydrobenz-1:4-thiazine-6-salicylic acid</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>5.7</td>
<td>No effect</td>
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</table>
TABLE of RESULTS (Contd)

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<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Solution</th>
<th>pH</th>
<th>Result</th>
</tr>
</thead>
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<tr>
<td>3-keto-2:3-dihydrobenz-1:4-thiazine-6-stibonic acid</td>
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<td>suspension</td>
<td>5.6</td>
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<td>emulsion</td>
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<td></td>
<td>1 in 2000</td>
<td></td>
<td>5.8</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td>1 in 1000</td>
<td></td>
<td>5.7</td>
<td>No effect</td>
</tr>
<tr>
<td>3-keto-2:3-dihydro-6:7-dihydroxybenz-1:4-thiazine</td>
<td>1 in 1000</td>
<td>solution</td>
<td>5.6</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td>1 in 1000</td>
<td>*solution + 0.05%</td>
<td>5.8</td>
<td>Very slight depressant effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot;Grill No.10&quot;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results given in the above table were obtained using 'intermediate' Ascaris preparations.
The "Crill No. 10" was added because it was thought that such a wetting agent might facilitate the passage of the test solution through the cuticle of the Ascaris preparation. From the results it will be seen that the presence of the "Crill No. 10" has brought about the desired effect. (Compare tracings 14 and 15). The "Crill No. 10" itself has no effect on the Ascaris preparation.

It was only possible to test a number of the prepared compounds against Ascaris, as such material was in short supply for long periods during the investigation.

RESULTS OBTAINED WITH LIVER FLUKE

Tracing No. 16

Compound: 3-Keto-2:3-dihydrobenz-1:4-thiazine
Concentration: 1 in 10,000 emulsion
pH: 7.0
Type and size of fluke: bovine, 2.5 cm.
Compound added after: 17 minutes.
General action: no change.
Amphetamine sulphate added after: 62 minutes.
Reaction to amphetamine sulphate: Amplitude and frequency increased.
Conclusion: no effect.

Tracing No. 17

Compound: 3-Keto-2:3-dihydrobenz-1:4-thiazine
Concentration: 1 in 8000 emulsion
pH: 6.9
Tracing No. 17 (Contd)

Type and size of fluke:— bovine, 1.5 cm.

Compound added after:— 15 minutes.

General action:— rise in tone 2 minutes after addition of compound. Normal frequency and amplitude destroyed but slight irregular movements.

Amphetamine sulphate added after:— 66 minutes.

Reaction to amphetamine sulphate:— normal rhythmic movement restored.

Conclusion:— slight paralysant effect.

Tracing No. 18

Compound:— 3-Keto-2:3-dihydrobenz-1:4-thiazine

Concentration:— 1 in 6000 emulsion.

pH:— 6.8

Type and size of fluke:— bovine, 1.5 cm.

Compound added after:— 15 minutes

General action:— slight increase in tone with very little movement.

Amphetamine sulphate added after:— 58 minutes

Reaction to amphetamine sulphate:— initial contraction and normal rhythmic movement restored.

Conclusion:— almost completely paralysant.

Tracing No. 19

Compound:— 3-Keto-2:3-dihydrobenz-1:4-thiazine

Concentration:— 1 in 4000 emulsion.

pH:— 6.9

Type and size of fluke:— sheep, 2 cm.

Compound added after:— 15 minutes.
Tracing No. 19 (Contd)

General action:— rhythmic movement continued for 2 minutes then fall in tone during the next 4 minutes after addition of the compound. Rhythmic movement destroyed but frequent convulsive movements, low tone maintained.

Amphetamine sulphate added after:— 70 minutes.

Reaction to amphetamine sulphate:— immediate rise in tone and normal rhythmic movement restored.

Conclusion:— almost completely paralysant.

Tracing No. 20

Compound:— 3-Keto-2:3-dihydrobenz-1:4-thiazine
Concentration:— 1 in 3000 emulsion
pH:— 6.9

Type and size of fluke:— bovine, 2.5 cm.

Compound added after:— 16 minutes.

General action:— slight rise in tone for 3 minutes after addition of the compound. Normal rhythmic movement destroyed followed by gradual fall in tone and very small irregular movements.

Amphetamine sulphate added after:— 59 minutes.

Reaction to amphetamine sulphate:— sharp initial contraction and normal rhythmic movement restored.
Conclusion: almost completely paralysant.

Tracing No. 21

Compound: 3-Keto-2:3-dihydrobenz-1:4-thiazine.
Concentration: 1 in 2000 emulsion.
pH: 7.0
Type and size of fluke: bovine, 2.5 cm.
Compound added after: 13 minutes.
General action: very slight movement for 1 minute after addition of the compound then movement completely destroyed.
Gradual fall in tone.
Amphetamine sulphate added after: 57 minutes.
Reaction to amphetamine sulphate: initial contraction and rhythmic movement gradually restored.
Conclusion: completely paralysant.

Tracing No. 22

Compound: 6-Amino-3-Keto-2:3-dihydrobenz-1:4-thiazine.
Concentration: 1 in 1000 emulsion.
pH: 6.9
Type and size of fluke: bovine, 2.5 cm.
Compound added after: 17 minutes.
General action: normal movement completed destroyed, very gradual fall in tone.
Amphetamine sulphate added after: 65 minutes.
Reaction to amphetamine sulphate: only very slight movement 5 minutes after addition.
Conclusion: almost lethal.

Tracing No. 23

Compound: 6-Amino-3-keto-2,3-dihydrobenz-1,4-thiazine, hydrochloride.

Concentration: 1 in 3000 solution.

pH: 6.9

Type and size of fluke: bovine, 2 cm.

Compound added after: 13 minutes.

General action: movement immediately destroyed.

Amphetamine sulphate added after: 60 minutes.

Reaction to amphetamine sulphate: initial contraction then relaxation, normal rhythmic movement restored after 3 minutes.

Conclusion: completely paralysant.

Tracing No. 24

Compound: 6-Acetamino-3-keto-2,3-dihydrobenz-1,4-thiazine.

Concentration: 1 in 1000 suspension.

pH: 7.0

Type and size of fluke: bovine, 2.5 cm.

Compound added after: 14 minutes

General action: initial contraction followed by irregular movements and fall in tone. Practically no movement 15 minutes after addition of compound.
Amphetamine sulphate added after: - 59 minutes.

Reaction to amphetamine sulphate: - initial contraction followed by irregular movements.

Conclusion: - paralysant after 15 minutes.

Tracing No. 25

Compound: - 6-Fluoro-3-keto-2:3-dihydrobenz-1:4-thiazine.

Concentration: - 1 in 2000 suspension.

pH: - 7.0

Type and size of fluke: - bovine, 2 cm.

Compound added after: - 14 minutes.

General action: - immediate fall in tone and movement completely destroyed.

Amphetamine sulphate added after: - 69 minutes.

Reaction to amphetamine sulphate: - initial contraction, rhythmic movement gradually restored.

Conclusion: - complete paralysant.

Tracing No. 26

Compound: - 6-Chloro-3-keto-2:3-dihydrobenz-1:4-thiazine.

Concentration: - 1 in 8000 emulsion.

pH: - 7.0

Type and size of fluke: - bovine, 2.5 cm.

Compound added after: - 15 minutes

General action: - fall in tone and movement practically destroyed.

Amphetamine sulphate added after: - 65 minutes.
Tracing No. 26 (Contd)

Reaction to amphetamine sulphate:— small contraction, rhythmic movement gradually restored.

Conclusion:— paralysant.

Tracing No. 27

Compound:— 6-Iodo-3-keto-2:3-dihydrobenz-1:4-thiazine.
Concentration:— 1 in 5000 fine suspension.

pH:—7.0

Type and size of fluke:— bovine, 2 cm.

Compound added after:— 19 minutes

General action:— fall in tone, small irregular movements for 10 minutes after addition of compound, then movement completely destroyed.

Amphetamine sulphate added after:— 68 minutes.

Reaction to amphetamine sulphate:— immediate contraction and normal rhythmic movement restored.

Conclusion:— paralysant.

Tracing No. 28

Compound:— 6-Thiocyanato-3-keto-2:3-dihydrobenz-1:4-thiazine

Concentration:— 1 in 1000 suspension. pH:— 7.0

Type and size of fluke:— bovine, 2 cm.

Compound added after:— 15 minutes.

General action:— large initial contraction for 2 minutes followed by gradual relaxation, irregular movements.
Tracing No. 28 (Contd)
becoming very small 12 minutes after addition of compound but never completely destroyed.
Amphetamine sulphate added after: 67 minutes
Reaction to amphetamine sulphate: small irregular movements.
Conclusion: almost paralytic.

Tracing No. 29
Compound: 6-Triazo-3-keto-2:3-dihydrobenz-1:4-thiazine.
Concentration: 1 in 6000 emulsion
pH: 7.0
Type and size of fluke: bovine, 2 cm
Compound added after: 12 minutes
General action: initial contraction followed by relaxation, movement completely destroyed, slight fall in tone.
Amphetamine sulphate added after: 56 minutes.
Reaction to amphetamine sulphate: initial contraction, normal rhythmic movement restored immediately.
Conclusion: completely paralytic.

Tracing No. 30
Compound: 6-Nitro-3-keto-2:3-dihydrobenz-1:4-thiazine.
Concentration: 1 in 4000 emulsion.
pH: 6.8
Compound added after: 16 minutes.
General action: small initial contraction followed by gradual fall in tone, movement completely destroyed.

Amphetamine sulphate added after: 63 minutes.

Reaction to amphetamine sulphate: initial contraction, rhythmic movement gradually restored.

Conclusion: completely paralysant.

**Tracing No. 31**

Compound: 6-Nitroso-3-keto-2:3-dihydrobenz-1:4-thiazine.

Concentration: 1 in 6000 emulsion.

pH: 7.0

Type and size of fluke: bovine 1.5 cm.

Compound added after: 13 minutes.

General action: increase in tone falling gradually 4 minutes after addition of compound, irregular movements which are almost completely destroyed 27 minutes after addition of compound.

Amphetamine sulphate added after: 56 minutes.

Reaction to amphetamine sulphate: rhythmic movement restored immediately.

Conclusion: paralysant.

**Tracing No. 32**

Compound: 3-Keto-2:3-dihydrobenz-1:4-thiazine-6-thiol.
Tracing No. 32 (Contd)

Concentration: 1 in 1000 emulsion
pH: 6.8

Type and size of fluke: bovine, 2 cm

Compound added after: 13 minutes

General action: initial contraction followed by irregular movements for 10 minutes after which the movement is completely destroyed, gradual fall in tone.

Amphetamine sulphate added after: 55 minutes.

Reaction to amphetamine sulphate: small contraction followed by relaxation practically no movement.

Conclusion: almost lethal.

Tracing No. 33

Compound: 3-Ketoadihydrobenz-1;4-thiazine-6-arsenic acid.

Concentration: 1 in 4000 suspension.

pH: 7.0

Type and size of fluke: sheep, 2 cm

Compound added after: 17 minutes.

General action: increase in amplitude during 23 minutes after addition after which amplitude is considerably decreased, rhythmic movement unaltered.

Amphetamine sulphate added after: 68 minutes.

Reaction to amphetamine sulphate: rhythmic movement continued, amplitude
Conclusion: very slight depressant effect.

**Tracing No. 34**

**Compound:** 3-Keto-2:3-dihydrobenz-1:4-thiazine-6-stibonic acid

**Concentration:** 1 in 4000 suspension

**pH:** 6.9

**Type and size of fluke:** sheep, 2.5 cm.

**Compound added after:** 15 minutes.

**General action:** no effect

Amphetamine sulphate added after: 64 minutes

**Reaction to amphetamine sulphate:** immediate contraction, normal rhythmic movement continued.

**Conclusion:** no effect.

**Tracing No. 35**

**Compound:** 3-Keto-2:3-dihydrobenz-1:4-thiazine-6-mercuric chloride.

**Concentration:** 1 in 4000 suspension

**pH:** 6.8

**Type and size of fluke:** sheep, 2.5 cm.

**Compound added after:** 15 minutes

**General action:** sharp initial contraction followed by very slight fall in tone and irregular movements.

Amphetamine sulphate added after: 63 minutes.

**Reaction to amphetamine sulphate:** sharp initial contraction, rhythmic movement restored.

**Conclusion:** slight paralysant effect.
Tracing No. 36

Compound: 6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine → phloroglucinol

Concentration: 1 in 4000 suspension

pH: 6.8

Type and size of fluke: bovine, 2.5 cm.

Compound added after: 11 minutes

General action: no effect

Amphetamine sulphate added after: 49 minutes

Reaction to amphetamine sulphate: rhythmic movement unaltered

Conclusion: no effect.

Tracing No. 37

Compound: 6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine → salicylic acid

Concentration: 1 in 4000 suspension

pH: 5.9

Type and size of fluke: bovine, 2 cm.

Compound added after: 12 minutes

General action: movement less regular, amplitude slightly decreased

Amphetamine sulphate added after: 60 minutes

Reaction to amphetamine sulphate: initial contraction, rhythmic movement restored.

Conclusion: very slight depressant effect.

Tracing No. 38

Compound: 6,6'-bis-(3-Keto-2:3-dihydrobenz-1:4-thiazine)

Concentration: 1 in 4000 suspension

pH: 6.8

Type and size of fluke: sheep, 2.5 cm.

Compound added after: 18 minutes
<table>
<thead>
<tr>
<th>Compound.</th>
<th>Concentration</th>
<th>Solution Emulsion or Suspension</th>
<th>Type of fluke</th>
<th>pH</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-keto-2:3-dihydrobenz-1:4-thiazine.</td>
<td>l in 10,000</td>
<td>emulsion</td>
<td>bovine</td>
<td>7.0</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td>l in 8000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.9</td>
<td>slight paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 6000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.8</td>
<td>almost completely paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 4000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.9</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>l in 3000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.9</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>l in 2000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7.0</td>
<td>completely paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 1000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>l in 4000</td>
<td>emulsion</td>
<td>sheep</td>
<td>7.2</td>
<td>slightly paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 2000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7.0</td>
<td>paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 1000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.9</td>
<td>almost lethal</td>
</tr>
<tr>
<td>6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine hydrochloride</td>
<td>l in 4000</td>
<td>solution</td>
<td>bovine</td>
<td>7.0</td>
<td>paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 3000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.9</td>
<td>completely paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 2000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.9</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>l in 1000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>6-acetamino-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>l in 4000</td>
<td>emulsion</td>
<td>bovine</td>
<td>7.0</td>
<td>slight depressant effect</td>
</tr>
<tr>
<td></td>
<td>l in 1000</td>
<td>suspension</td>
<td>&quot;</td>
<td>7.0</td>
<td>paralysant after 15 minutes</td>
</tr>
<tr>
<td>6-fluoro-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>l in 4000</td>
<td>emulsion</td>
<td>sheep</td>
<td>6.8</td>
<td>slightly paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 2000</td>
<td>suspension</td>
<td>bovine</td>
<td>7.0</td>
<td>paralysant</td>
</tr>
<tr>
<td>6-chloro-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>l in 8000</td>
<td>emulsion</td>
<td>bovine</td>
<td>7.0</td>
<td>paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 6000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>6.9</td>
<td>completely paralysant</td>
</tr>
<tr>
<td></td>
<td>l in 4000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7.1</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>l in 1000</td>
<td>suspension</td>
<td>bovine</td>
<td>6.9</td>
<td>&quot;</td>
</tr>
<tr>
<td>Compound</td>
<td>Concentration</td>
<td>Solution or Suspension</td>
<td>Type of Fluke</td>
<td>pH</td>
<td>Result</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------</td>
<td>------------------------</td>
<td>---------------</td>
<td>-------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>6-ido-3-keto-2:3-</td>
<td>1 in 6000</td>
<td>suspension</td>
<td>bovine</td>
<td>7.0</td>
<td>paralysant</td>
</tr>
<tr>
<td>6-thiocyan-3-keto-2:3-dihydro</td>
<td>1 in 4000</td>
<td>emulsion</td>
<td>bovine</td>
<td>6.9</td>
<td>completely paralysant</td>
</tr>
<tr>
<td>benz-1:4-thiazine</td>
<td>1 in 1000</td>
<td>suspension</td>
<td>sheep</td>
<td>6.1</td>
<td>no effect</td>
</tr>
<tr>
<td>6-triaz-3-keto-2:3-</td>
<td>1 in 8000</td>
<td>emulsion</td>
<td>bovine</td>
<td>6.9</td>
<td>slightly paralysant</td>
</tr>
<tr>
<td>dihydrobenz-1:4-thiazine</td>
<td>1 in 6000</td>
<td>suspension</td>
<td>sheep</td>
<td>7.0</td>
<td>completely paralysant</td>
</tr>
<tr>
<td>6-nitro-3-keto-2:3-</td>
<td>1 in 4000</td>
<td>emulsion</td>
<td>bovine</td>
<td>6.9</td>
<td>slightly paralysant</td>
</tr>
<tr>
<td>dihydrobenz-1:4-thiazine</td>
<td>1 in 1000</td>
<td>suspension</td>
<td>sheep</td>
<td>6.8</td>
<td>completely paralysant</td>
</tr>
<tr>
<td>6-nitroso-3-keto-2:3-</td>
<td>1 in 6000</td>
<td>emulsion</td>
<td>bovine</td>
<td>7.0</td>
<td>completely paralysant</td>
</tr>
<tr>
<td>dihydrobenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>sheep</td>
<td>7.0</td>
<td>completely paralysant</td>
</tr>
<tr>
<td>3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>emulsion</td>
<td>bovine</td>
<td>7.0</td>
<td>slightly paralysant</td>
</tr>
<tr>
<td>6-thiol</td>
<td>1 in 1000</td>
<td>suspension</td>
<td>sheep</td>
<td>6.9</td>
<td>almost lethal</td>
</tr>
<tr>
<td>3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>sheep</td>
<td>7.0</td>
<td>slight depressant</td>
</tr>
<tr>
<td>6-arsenic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>effect</td>
</tr>
<tr>
<td>3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>sheep</td>
<td>6.9</td>
<td>no effect</td>
</tr>
<tr>
<td>Compound</td>
<td>Concentration</td>
<td>Solution</td>
<td>Type of Fluke</td>
<td>pH</td>
<td>RESULT</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>---------------</td>
<td>----------</td>
<td>---------------</td>
<td>-----</td>
<td>-------------------------</td>
</tr>
<tr>
<td>5-keto-2:3-dihydrobenz-1:4-thiazine-6-mercuric chloride</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>sheep</td>
<td>6.8</td>
<td>slight paralysant effect</td>
</tr>
<tr>
<td>6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine-6-phloroglucinol</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>bovine</td>
<td>6.8</td>
<td>no effect</td>
</tr>
<tr>
<td>6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine-&gt;salicylic acid</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>bovine</td>
<td>5.9</td>
<td>slight depressant effect</td>
</tr>
<tr>
<td>5:6-bis-(3-keto-2:3-dihydrobenz-1:4-thiazine)</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>sheep</td>
<td>6.8</td>
<td>no effect</td>
</tr>
<tr>
<td>5-keto-2:3-dihydro-5:7-dimethoxybenz-1:4-thiazine</td>
<td>1 in 6000</td>
<td>emulsion</td>
<td>bovine</td>
<td>6.9</td>
<td>slight depressant effect</td>
</tr>
<tr>
<td>5-keto-2:3-dihydro-5:7-dihydroxybenz-1:4-thiazine</td>
<td>1 in 5000</td>
<td>&quot;</td>
<td>sheep</td>
<td>7.0</td>
<td>paralysant after 29 mins</td>
</tr>
<tr>
<td>5-keto-2:3-dihydro-5:7-dihydroxybenz-1:4-thiazine</td>
<td>1 in 4000</td>
<td>suspension</td>
<td>bovine</td>
<td>7.0</td>
<td>completely paralysant</td>
</tr>
<tr>
<td>5-keto-2:3-dihydro-5:7-dihydroxybenz-1:4-thiazine</td>
<td>1 in 1000</td>
<td>solution</td>
<td>sheep</td>
<td>6.8</td>
<td>slight depressant effect</td>
</tr>
<tr>
<td>5-keto-2:3-dihydro-5:7-dihydroxybenz-1:4-thiazine</td>
<td>1 in 2000</td>
<td>&quot;</td>
<td>bovine</td>
<td>6.9</td>
<td>depressant effect</td>
</tr>
<tr>
<td>5-keto-2:3-dihydro-5:7-dihydroxybenz-1:4-thiazine</td>
<td>1 in 1000</td>
<td>emulsion</td>
<td>&quot;</td>
<td>7.0</td>
<td>completely paralysant</td>
</tr>
</tbody>
</table>
In order to compare the effect of the various compounds tested with liver fluke the Threshold Value was determined. This value was arrived at in the following way.

The compound, if completely paralysant at a particular concentration, was tested at various dilutions until it had no effect on the liver fluke preparation. The minimum concentration at which complete paralysis was produced within 45 minutes was taken as the concentration from which the Threshold Value was derived. The test was repeated with at least four flukes at this concentration. Thus, if a concentration of 1 in 5000 produced complete paralysis within 45 minutes, the compound was said to have a Threshold Value of 5000.

This point is illustrated in Tracings 16 to 21 which show the effect of 3-keto-2:3-dihydrobenz-1:4-thiazine at concentrations ranging from 1 in 10,000 to 1 in 2000. At a concentration of 1 in 10,000 (Tracing 16) there is no effect; at 1 in 3000 (Tracing 20) there is almost complete paralysis and at 1 in 2000 (Tracing 21) complete paralysis is produced within 45 minutes of addition of the compound. The unsubstituted compound is therefore said to have a Threshold Value of 2000.

The Threshold Values of the compounds tested are shown in the following table.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Threshold Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Chloro-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>3000</td>
</tr>
<tr>
<td>6-Triazo-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>6000</td>
</tr>
<tr>
<td>6-Nitroso-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>6000</td>
</tr>
<tr>
<td>6-Iodo-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>5000</td>
</tr>
<tr>
<td>6-Nitro-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>4000</td>
</tr>
<tr>
<td>3-Keto-2:3-dihydro-6:7-dimethoxybenz-1:4-thiazine</td>
<td>4000</td>
</tr>
<tr>
<td>6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine, hydrochloride</td>
<td>3000</td>
</tr>
<tr>
<td>3-Keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>2000</td>
</tr>
<tr>
<td>6-Fluoro-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>2000</td>
</tr>
<tr>
<td>6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1000</td>
</tr>
<tr>
<td>6-Acetamino-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1000</td>
</tr>
<tr>
<td>6-Thiocarbonyl-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>1000</td>
</tr>
<tr>
<td>3-Keto-2:3-dihydrobenz-1:4-thiazine-6-thiol</td>
<td>1000</td>
</tr>
<tr>
<td>3-Keto-2:3-dihydro-6:7-dihydroxybenz-1:4-thiazine</td>
<td>1000</td>
</tr>
<tr>
<td>3-Keto-2:3-dihydrobenz-1:4-thiazine-6-arsonic acid</td>
<td>&lt; 4000</td>
</tr>
<tr>
<td>3-Keto-2:3-dihydrobenz-1:4-thiazine-6-stibonic acid</td>
<td>&lt; 4000</td>
</tr>
<tr>
<td>3-Keto-2:3-dihydrobenz-1:4-thiazine-6-mercuric chloride</td>
<td>&lt; 4000</td>
</tr>
<tr>
<td>6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine-6-phloroglucinol</td>
<td>&lt; 4000</td>
</tr>
<tr>
<td>6-Amino-3-keto-2:3-dihydrobenz-1:4-thiazine-6-salicylic acid</td>
<td>&lt; 4000</td>
</tr>
<tr>
<td>6:6'-bis-(3-Keto-2:3-dihydrobenz-1:4-thiazine)</td>
<td>&lt; 4000</td>
</tr>
</tbody>
</table>
These six compounds formed suspensions at 1 in 4000 and therefore could not be tested at higher concentrations. It is possible, however, had they been more soluble, that a Threshold Value could have been obtained.

The results of the tests carried out in the Department of Zoology at the University of Edinburgh on the free living stages of Bursate nematodes in faecal cultures are given in the following table and compared with the results obtained with Baldwin's technique for these compounds.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Percentage compound required for 90% Kill</th>
<th>Percentage compound required for 99% Kill</th>
<th>Threshold Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>5.0</td>
<td>25.0</td>
<td>2000</td>
</tr>
<tr>
<td>5-amino-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>no lethal value</td>
<td>no lethal value</td>
<td>1000</td>
</tr>
<tr>
<td>6-amino-3-keto-2:3-dihydrobenz-1:4-thiazine, hydrochloride</td>
<td>7.5</td>
<td>25.0</td>
<td>3000</td>
</tr>
<tr>
<td>6-chloro-3-keto-2:3-dihydrobenz-1:4-thiazine</td>
<td>&gt;50.0</td>
<td>no lethal value</td>
<td>8000</td>
</tr>
<tr>
<td>3-keto-2:3-dihydro-6:7-dimethoxybenz-1:4-thiazine</td>
<td>50.0</td>
<td>&gt;50.0</td>
<td>4000</td>
</tr>
</tbody>
</table>
DISCUSSION OF RESULTS

The results obtained in the investigation present several points for discussion. The main fact which has arisen is that 3-keto-2:3-dihydrobenz-1:4-thiazine and its derivatives, which have so far been fully tested in vitro, produce a paralysant effect on liver fluke as shown in the experiments, whilst no effect is observed with Ascaris. Observations have been made on derivatives substituted in the 6- and 6:7- positions.

The effects of these compounds on Ascaris and liver fluke will be discussed together under the headings of the substituent groups as follows:

(1) Introduction (2) Unsubstituted compound
(3) 6-Amino-derivative (4) 6-Amino hydrochloride
(5) 6-Acetamino-derivative (6) Halogen derivatives
(7) Thiocyanate-derivative (8) Triazo-derivative
(9) Nitro-derivative (10) Nitroso-derivative
(11) Thiol-derivative (12) 6:7-Dimethoxy-derivative
(13) 6:7-Dihydroxy-derivative (14) Insoluble derivatives giving suspensions at a concentration of 1 in 4000 (15) General remarks.

(1) INTRODUCTION

3-Keto-2:3-dihydrobenz-1:4-thiazine and its derivatives all contain a ketonic group which Baldwin (Brit. J. Pharmacol., 1948, 2, 95) has shown to possess potential anthelmintic properties. It would appear also (Baldwin, loc. cit., 94) that an
unsaturated ring may contribute to anthelmintic potency. In the compounds under consideration an unsaturated ring is present. This, however, is a condensed ring, which Baldwin (loc. cit., 101) has pointed out to be less effective than a separated ring.

The above may be contributory factors as far as the action of the compounds on liver fluke is concerned.

The inactivity of the benz-thiazine derivatives, with the exception of the unsubstituted and its 6-nitroso derivative, towards Ascaris may be due to difficulty of penetration of the Ascaris cuticle. It may, however, be possible to overcome such a difficulty by the use of an efficient wetting agent. This point is illustrated in the case of the 6:7-dihydroxy-derivative which was tested alone, and in the presence of "Grill No.10", which is an extremely efficient wetting agent. It will be seen from a study of Tracings 14 and 15, that in the presence of the "Grill No.10", the dihydroxy-derivative has a slight depressant effect which is not present when the compound is tested alone. The effect, however, is very slight but it does show the possibility of using wetting agents to assist in the penetration of the Ascaris cuticle.

Trim (Parasitol, 1949, 39, 285) found that certain surface active agents were able to produce an acceleration of the rate of penetration of the
compound through the Ascaris cuticle. Compounds such as cetyl trimethyl ammonium bromide and sodium cetyl sulphate, which are cationic and anionic detergents respectively, accelerated the rate of penetration of nicotine and hexyl resorcinol into the Ascaris whilst sodium tauroglycocholate had no effect. Further effects of this kind were observed by Seelkopf and Auterhoff (Pharmazie, 1950, 5,463). They found that the activity of many substances was increased, when tested in vitro with Ascaris, in the presence of Pel Tauri (ox bile). The increases were greater than when saponin or "Tween" preparations were used, the latter being similar in composition to "Crill No.10".

It is agreed that the use of Ascaris preparations, where only a small portion of the helminth is used, may not be entirely satisfactory, as this can only show the effect of the compound on the neuro-muscular system of the helminth and prevents the compound entering the alimentary canal. Baldwin (loc.cit.92) states, however, that the whole Ascaris is not reliable for in vitro testing.

(2) UNSUBSTITUTED COMPOUND

The unsubstituted compound has a slight stimulant effect on Ascaris at 1 in 4000 and a paralysant effect on liver fluke at 1 in 2000 thus giving it a Threshold Value of 2000. It would appear, therefore, that this compound is capable of penetrating the Ascaris cuticle and stimulates the musculature.
The amino-derivative has no effect on *Ascaris* but completely paralyses the liver fluke at a concentration of 1 in 1000, at which the effect is almost lethal. This compound, Threshold Value 1000, therefore, has approximately half the anthelminthic potency of the unsubstituted compound i.e. the amino group lowers the activity.

This compound has no effect on *Ascaris* but is three times (Threshold Value 3000) as active as the free base (Threshold Value 1000) towards liver fluke. This is probably due to free acid in solution. Comparison of the Threshold Values in this case is not strictly correct as the hydrochloride was tested in solution whilst the amino-compound was tested as an emulsion.

Acetylation did not change the effect of the amino-derivative against *Ascaris* nor the effect on liver fluke, since the Threshold Value of the latter was the same as the free base. This is perhaps surprising, as acetylation might be expected to produce some change in the potency.

The halogen derivatives tested have no effect on *Ascaris*. This is unexpected, as a number of organic chlorine compounds are active anthelmintics e.g. tetrachloroethylene is effective against
Ascaris and other nematodes.

They all, however, have a paralysant effect on liver fluke, the Threshold Values being fluoro 2000, chloro 3000 and iodo 5000. The iodo-compound formed a fine suspension at 1 in 5000 so that the Threshold Value is probably slightly higher than 5000. It would appear that there is no correlation between the halogens; chloro, the middle member of the series having a greater potency than both the fluoro and iodo compounds. It may be that a maximum is reached with chlorine, this however is only a tentative suggestion as the bromo-compound has not been tested. On the other hand it was found in the case of the toxicity of D.D.T. analogues to lice (W.A. Sexton, Chemical Constitution and Biological Activity, London, 1949, p. 314) that the compounds Cl\[CH\cdot(CCl)\cdotCl\] and F\[CH\cdot(CCl)\cdotF\] had an equal effect. Therefore the effect of the addition of the halogens may depend on the composition of the molecule to which the addition takes place. Williams, Schelling and Hartman (Amer. J. Trop. Med., 1949, 29, 241) state that the anthelmintic activity of a compound may be increased by the introduction of certain halogens.

In the case of 3-keto-2:3-dihydrobenz-1:4-thiazine, introduction of fluorine has no effect on the anthelmintic potency whilst both chlorine and iodine increase the potency considerably, 6-chloro-3-keto-2:3-dihydrobenz-1:4-thiazine being
the most potent of the compounds tested in this investigation.

(7) THICOL-derivative

Introduction of the C6H5-group gives a compound which has no effect on Ascaris but has a paralyzing effect on liver fluke at a concentration of 1 in 1000. Thus it appears that the C6H5-group lowers the anthelmintic activity by approximately one half.

(8) TRIAZO-derivative

The triazo-derivative has no effect on Ascaris but has a strong effect on liver fluke, having a Threshold Value of 6000, indicating that the anthelmintic potency has been increased three times by the introduction of the double bond.

(9) NITRO-derivative

This compound has no effect on Ascaris but has double the effect of the unsubstituted compound on liver fluke, the Threshold Value being 4000.

(10) NITROSO-derivative

The nitroso-compound behaves differently towards Ascaris and liver fluke. At a concentration of 1 in 4000 it has a slight stimulant effect on Ascaris whilst at 1 in 6000 it completely paralyzes liver fluke. The reverse of this action was noted by Chance and Mansour (Brit. J. Pharmacol., 1949, 4, 12) in the case of coumarin which was paralytic to Ascaris and stimulant to liver fluke. The introduction of the NO-group into the unsubstituted compound does not destroy the slight stimulant effect on
Ascaris but increases the potency towards liver fluke approximately three times.

It is interesting to note that the nitroso-group is 1.5 times as active towards liver fluke as the nitro-group.

(11) THIOL-derivative

From the results it will be seen that the thiol-derivative has no effect on Ascaris but paralyses liver fluke at a concentration of 1 in 1000. Therefore, the introduction of the SH-group halves the activity of the unsubstituted compound.

(12) 6:7-DIMETHOXY-derivative

The 6:7-dimethoxy-derivative, although having no effect itself on Ascaris, has removed the slight stimulant effect of the unsubstituted compound. This fact may give some support to Baldwin's observation (loc.cit.95) that certain alkylolxy-groups can increase the anthelmintic activity.

The anthelmintic activity is also increased with liver fluke by the introduction of the methoxy-groups, the effect being approximately doubled, the Threshold Value rising from 2000 in the case of the unsubstituted to 4000 for the 6:7-dimethoxy-compound.

(13) 6:7-DIHYDROXY-derivative

Although many phenols possess anthelmintic properties towards Ascaris e.g. \( \beta \)-naphthol, no activity was found with the 6:7-dihydroxy-derivative. It has been observed, however, that the position of
of the hydroxyl group in phenols is an important factor, e.g. Baldwin (loc. cit. 102) found that 4-benzylphenol was three times as active as the 2-derivative. The presence of two hydroxyl groups in the benz-thiazine derivative does not appear to have any effect. The n-alkyl resorcinols, on the other hand, possess anthelmintic properties, but the 6:7-dihydroxy-compound is not an exactly parallel case as the hydroxyl groups are in the ortho-position to one another.

The 6:7-dihydroxy-compound although paralysing the liver fluke at a concentration of 1 in 1000 has a lower Threshold Value than the unsubstituted compound, thus it would appear that the introduction of the two hydroxyl groups into the benz-thiazine molecule lowers the anthelmintic potency. This is most unexpected as many cases are known where the presence of hydroxyl groups increases the activity.

(14) Insoluble derivatives giving suspensions at a concentration of 1 in 4000

The compounds falling into this class which were tested against Ascaris are the 6-arsenic and 6-stibonic acid derivatives, both of which have no effect on the Ascaris preparation.

Liver fluke were tested with six insoluble derivatives, namely, the 6-arsenic and stibonic acids, the 6-mercuric chloride compound, the 6-amino compound coupled with phloroglucinol and salicylic acid, and 6:6'-bis-(3-keto-2:3 dihydrobenz-1:4-thiazine). Of these, only three have any effect.
3-keto-2:3-dihydrobenz-1:4-thiazine-6-arsenic acid, 3-keto-2:3-dihydrobenz-1:4-thiazine-6-mercuric chloride and 6-amin0-3-keto-2:3-dihydrobenz-1:4-thiazine ——> salicylic acid all having a slight depressant effect.

It is possible, had these compounds been more soluble, that they would have had a much greater effect on Ascaris and liver fluke at higher concentrations.

(15) GENERAL REMARKS

If we consider the effect of the individual atoms or groups on the anthelmintic activity in the case of Ascaris, it will be seen that the groups introduced, with the exception of the nitroso-group, all destroy the slight stimulant effect of the unsubstituted compound but they were not sufficiently potent to produce even a depressant effect on the Ascaris preparation. If penetration of the Ascaris cuticle does not take place, the compounds will have no effect, and the Ascaris preparation will continue its normal movement, so giving the impression that the effect of the unsubstituted compound has been destroyed.

The effect of the individual atoms or groups on liver fluke is much more definite and is most conveniently discussed by consideration of the Threshold Value.

The unsubstituted 3-keto-2:3-dihydrobenz-1:4-thiazine has a Threshold Value of 2000. The introduction of the following groups Cl, N3−, NO−, I, NO2−,
6:7-dimethoxy and \(-\text{NH}_2\cdot\text{HCl}\) increase this value whilst \(-\text{NH}_2, \text{CH}_3\text{CONH}-, \text{CNS}-, \text{SH}-\) and 6:7-dihydroxy decrease this value, fluorine having no effect. From this we may conclude that the anthelmintic potency of 3-keto-2:3-dihydrobenz-1:4-thiazine is increased towards liver fluke by the presence of \(\text{Cl}, \text{N}_3-, \text{NO}_2-, \text{I} \) and \(\text{NO}_2-\) in the 6-position and \(-\text{CH}_3\) in the 6 and 7-positions whilst \(-\text{NH}_2, \text{CH}_3\text{CONH}-, \text{CNS}-\) and \(\text{SH}-\) in the 6- position and \(-\text{OH}\) in the 6 and 7-positions reduce the activity by approximately one half.

The atoms or groups may then be arranged in the following order of potency:

\[
\text{Cl} > -\text{N}_3, \text{NO}_2 > \text{I} > -\text{NO}_2, \text{6:7-dimethoxy} > -\text{NH}_2 \text{HCl} > \text{unsubstituted}, \text{F} > -\text{NH}_2, \text{CH}_3\text{CONH}-, \text{CNS}-, \text{SH}-\) and 6:7-dihydroxy.
\]

Filicic acid, the most important organic acid of filix mas, which contains the \(-\text{CO-NH}_2-\) grouping also present in the benz-thiazine derivatives tested, was found by Chance and Mansour (loc.cit.10) to have a lethal action on liver fluke at a concentration of 1 in 5000. Thus, it would appear, that the above mentioned grouping has a definite anthelmintic activity towards liver fluke although in the case of the benz-thiazine derivatives the activity is considerably less than in filicic acid.

Comparison of the results of the compounds tested against the free living stages of Bursate nematodes in faecal cultures with those obtained
with liver fluke show that there is no similarity of action between the two types of nematode preparation, and the five compounds tested would appear to have no practical larvaecidal value.
SUMMARY and CONCLUSION

It may be concluded that 3-keto-2:3-dihydrobenz-1:4-thiazine and its derivatives, with the exception of the unsubstituted compound itself and the 6-nitroso-derivative both of which have a slight stimulant effect, have no effect on the 'intermediate' preparations of Ascaris.

All but six, namely the 6-azetriconic- and stibonic acids, the 6-mercuric chloride compound, the 6-amino compound coupled with phloroglucinol and salicylic acid, and 6:6'-bis-(3-keto-2:3-dihydrobenz-1:4-thiazine), have a paralysant effect on liver fluke, the 6-amino and 6-thiol compounds being almost lethal at a concentration of 1 in 1000.

Of the compounds tested the 6-chloro-3-keto-2:3-dihydrobenz-1:4-thiazine is the most potent, having an activity four times that of the unsubstituted compound.

We may, therefore, conclude that certain atoms or groups increase the activity, whilst others decrease the activity of 3-keto-2:3-dihydrobenz-1:4-thiazine towards liver fluke. The inactivity towards Ascaris may be partly due to the inability of the compounds to penetrate the Ascaris cuticle, a difficulty which it might be possible to overcome by using a suitable wetting agent.
ACKNOWLEDGMENTS

The Author is indebted to the Agricultural Research Council for the grant which made the investigation possible, to Mr. Soulsby, City of Edinburgh Abattoir, Slateford, for supplies of parasites used during the investigation, to Dr. Minnis and Mr. A.T. Macdonald who carried out the micro-chemical analysis.
TRACINGS.