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THE TOXICITY AND PHARMACOLOGY OF LICHENIFORMIN A5

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Growth of the N.C.T.C. 7072 of *B. licheniformis* on a synthetic ammonium-lactate medium (Belton, Hills, and Powell, 1949) gives rise to antibiotic material which has been named licheniformin (Callow, Glover, D'Arcy Hart, and Hills, 1947). The A5 strain of *B. licheniformis* on a glucose-ammonium-lactate medium (Hills, Belton, and Blatchley, 1949) gives rise to another antibiotic material which has been called ayfivin (Arriagada, Savage, Abraham, Heatley, and Sharp, 1949). If the A5 strain is grown on the ammonium-lactate medium of Callow *et al.*, antibiotic material is produced which is a mixture of polypeptides resembling licheniformin and for which the provisional name of licheniformin A5 is proposed until further investigation shows whether or not it is identical with licheniformin.

This communication gives the results of some biological observations on licheniformin A5 hydrochloride and two fractions of it.

METHODS

Materials

The antibiotic material was isolated from the culture medium by absorption on Decalso F, elution with 10 per cent (w/v) sodium chloride solution, precipitation as picrate, and conversion to hydrochloride. The solid product from 18 batches representing 2,300 litres of culture was bulked and referred to as licheniformin A5 (DB.1). This polypeptide mixture contained the total biologically active material of the cultures. It can be separated into fractions by fractional elution from an acid washed alumina column at controlled *p*H. The fraction eluted by 10 per cent (v/v) methanol in ethanol is referred to as DEA.1, and that eluted by 50 per cent (v/v) methanol-water as DEA.2.

The materials used were prepared for us by our colleagues L. H. Kent and B. T. Tozer. A description of the methods of isolation is in course of preparation.

Dilution assay

The biological activity of licheniformin A5 was expressed in units based on the inhibition of *M. phlei*, strain N.C.T.C. 525, after 48 hours' incubation in tryptic beef broth (0.5 per cent (w/v) N) at 37° C. The technique used was that described by Callow *et al.* (1947) and by Belton *et al.* (1949) in which the antibiotic is serially diluted in 2 ml. amounts of broth in sterile test-tubes, and a very small piece of *M. phlei* pellicle is floated on the surface of the medium in each tube. Final results were obtained by testing in triplicate with 50 per cent differences between dilutions, when potency ratios less than 1.5 were not significant.

The end–point chosen was not complete inhibition of growth, but the presence of easily visible growth extending over not more than a quarter of the surface. The pooled material DB.1 was shown to produce this degree of inhibition in a dilution of 1 part in 6×10^6. It was therefore deemed to have 6×10^6 units per g. (1 unit = 0.166 μg.), and estimations of the activity of unknown materials were carried out in comparison with this standard. Solutions (100 mg./ml. distilled water) showed no loss in biological activity during storage at *p*H 3 and 6.5, and at 0° C. and 25° C. for a period of 120 days, in a highly replicated test such that the limits for a significant difference P = 0.95 were 74–134 per cent.

The biological activity of fraction DEA.1 per unit mass was 1.8 times greater than that of the crude material, but the activity of fraction DEA.2 was only half as great as that of the crude material.

Assays of licheniformin A5 in the blood and tissues of experimental animals were carried out on pooled samples from groups of ten animals. Normal blood and tissue suspensions did not affect the end-point. To facilitate the homogenizing of the samples of tissues, they were lyophilized, then subsequently ground and suspended in sterile distilled water.

Acute Toxicity for Animals

Some species of laboratory animals were injected by the three common parenteral routes. Table I shows the weighted means and fiducial limits (P = 0.95) of the LD50 doses in mice, together with the average values obtained from a few tests in guinea-pigs and rats.

TABLE I

THE LD50 DOSES OF LICHENIFORMIN A5

Route	Mouse (20 g.)		Guinea-pig (300 g.)	Rat (200 g.)
	Weighted mean	Fiducial limits		
Subcutaneous mg.	13.4	11.73–14.37	200	60
mg./kg.	670	587–719	670	300
Intraperitoneal mg.	9.52	9.14–9.92	150	
mg./kg.	476	457–496	500	
Intravenous mg.	7.36	7.11–7.61	30	
mg./kg.	368	356–381	100	

When the drug is injected subcutaneously the rat is twice as sensitive as the mouse and guinea-pig. Of the two latter species the guinea-pig is much more sensitive than the mouse to intravenous injections.

After subcutaneous injection of the LD50 dose there is a short transient period of depression followed by absence of any symptoms for a day or two until an increasing degree of weakness and prostration terminates fatally between the second and fourth day. Intravenous injections in the lethal range produce the collapse and death of the animal instantaneously, or very shortly after the completion of the injection. This syndrome was not affected by adjustment of the *p*H of the drug-solution within the range 6.6–8.2, or by the inclusion of calcium lactate in the injections to the maximal degree tolerated by mice.

The acute toxicity of licheniformin A5 for the mouse by the subcutaneous route (670 mg./kg.) is of the same order as that of licheniformin and ayfivin, but

is about twice that of streptomycin (1,250 mg./kg.) and five times that of aureomycin (3,000–4,000 mg./kg.). Intravenously in the mouse, licheniformin A5 is among the least toxic of the common antibiotics, being slightly less toxic than licheniformin, ayfivin, chloromycetin, and some samples of streptomycin. It is several times less toxic than aureomycin by this route.

PHARMACOLOGY

Concentration in blood

Mice were used in experiments to determine the concentration of licheniformin A5 occurring in the blood, kidneys, and other tissues after single and repeated subcutaneous injections in saline. As will be seen from Fig. 1, single doses resulted

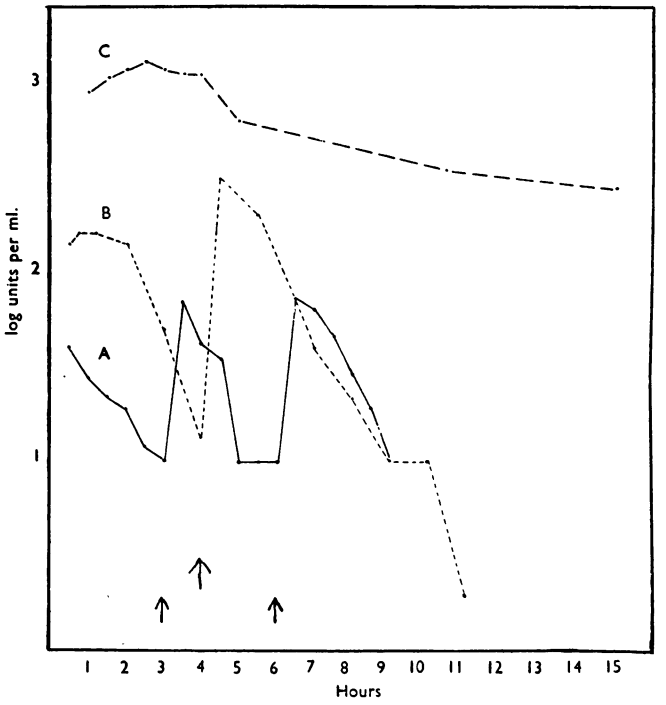

FIG. 1.—Licheniformin A5 concentrations in the blood of the mouse after subcutaneous injections. (A) 0.5 mg. per mouse, three doses at 3-hourly intervals. (B) 1.0 mg. per mouse, repeated after 4 hours. (C) 10.0 mg. per mouse.

in an early peak concentration in the blood followed by a fairly rapid fall which was especially steep with small doses such as 1 mg. It will be noted that a large dose such as 10 mg. is relatively persistent in the blood and 90 per cent of the maximum is still in circulation at the 6th hour, whereas, after a dose of 1 mg., only 8 per cent of the maximum remains after four hours and it is just detectable after five hours. Graphs A and B of Fig. 1 also show that a second injection, if given as the effect of the first injection is waning, immediately raises the blood level to a concentration nearly double that from the single dose. Further injections do not result in the peak being raised any higher, and as a steep fall occurs in the blood level after each peak the repeated injections simply restore temporarily the maximal blood

concentration achieved by the second dose, and also gradually prolong the persistence of drug at its post-maximal levels before excretion.

Distribution in body

The extent to which the drug injected subcutaneously became dispersed in the body of the mouse was determined by assaying the blood, lungs, liver, kidneys, and spleen at various periods after the time of dosing. It was found that the antibiotic, apart from its fleeting appearance in the blood, was very largely to be found in the kidneys and urine.

Quantitative testing of mouse urine was not possible because of lack of material, but the small amounts of urine available were shown to cause *in vitro* inhibition of the test organism, and from the fact that the kidney alone of the body tissues significantly takes up the licheniformin A5 from the blood, as the level in the latter falls, it can be safely deduced that excretion is mainly in the urine.

After a dose of 1 mg. a typical residual distribution in the tissues was kidneys 96 per cent, liver 2–4 per cent, and lungs 0.1–0.2 per cent. If the relative concentrations in the blood and kidneys were determined frequently—commencing 30 minutes after a dose of 1 mg.—it was found (Table II) that only within the first hour did the blood contain more drug than the kidneys, and thereafter as the level in the blood fell to zero the concentration in the kidneys rose rapidly to a maximum.

TABLE II

THE RELATIVE DISTRIBUTION OF LICHENIFORMIN A5 BETWEEN BLOOD AND KIDNEYS OF THE MOUSE
Dose: 1 mg. (6,000 units) subcutaneously

Time in hours :	½	1	1½	2	3	3½	4	5	6	15
Blood; units/2 ml.	260	300	230	180	120	55	30	2	0	0
Kidneys; units/2 kidneys ..	160	300	400	770	1,880	1,780	1,300	1,780	1,600	1,780
Percentage in blood relative to kidneys	62	50	37	19	6	3	2	0.1	0	0
Percentage in kidneys relative to blood	38	50	63	81	94	97	98	99.9	100	100

This concentration in the kidney persisted for some hours and then slowly decreased during succeeding weeks.

In eight experiments in which the mice had been kept for 2–3 weeks after having had a single dose or a series of doses, the activity per gramme of kidney tissue was found to be 40–60 per cent of the peak level, with an average value of 52 per cent. In three of the above experiments some mice of the group were kept 12–15 weeks after their last dose, and the residual activities in the kidney tissue relative to the peak concentration were respectively 13, 16, and 18 per cent.

As with the blood levels, the maximum in the kidney from a small dose such as 1 mg. was reached earlier than from a large dose such as 10 mg., the kidney maxima being reached in 3 and 6 hours respectively. The maximal concentration reached in the kidney tissue, and therefore the concentration remaining for a period subsequently, was with both large and frequently repeated doses less than proportional to the number of units injected. Thus an increase in the dose from 1 to 10 mg.

only raised the concentration in the kidney tissue fivefold. Six doses of 1 mg. at three-hourly intervals produce a tissue level only three times greater than that from one dose, and the increase from twelve doses was only sixfold.

When mice were dosed orally with 10 mg., the resulting peak blood-level made a transient appearance an hour later and was extremely low, being only 20 units/ml. compared with the value of 1,300 reached after a subcutaneous injection. By the latter route also this dose caused a very marked cellular reaction in the kidneys, whereas the oral dosing caused no detectable change.

Oral dosing is therefore a wholly unsatisfactory way of attempting to achieve a general distribution of licheniformin A5 in the body.

Nephrotoxicity

The nature and extent of the cellular changes in the kidney resulting from single and repeated subcutaneous doses of licheniformin A5 were intensively studied in the mouse, and some comparative results were also obtained from the guinea-pig, rat, and rabbit.

Mouse

Histological examination was made of the kidneys of all mice killed for biological assay of the licheniformin A5 level in blood and organs.

It was established at the outset that the kidneys were the only organs showing specific changes. These specific changes vary in degree with the dose administered, and after any one dose the degree of change increases from hour to hour up to 20–30 hours after the dose. With low dosage return to normal is complete, with larger doses there is permanent change in the kidneys.

At first haemalum eosin preparations were studied. The earliest, and with low dosage often the only, change was increased eosinophilia of the epithelium of the first part of the nephron (the proximal convoluted tubules). Larger doses brought about a change in the cells where eosinophilic granules were visible and sometimes large eosinophilic globules were found in the epithelial cells or free in the lumen. These changes were entirely confined to those epithelial cells normally showing a basal palisade of mitochondria and a brush border lining the lumen—i.e., proximal convoluted tubules in the cortex and its terminal straight portion in the cortico-medullary zone (subcortical zone). When special stains for mitochondria were used these grades of change were found to indicate a progressive alteration in the mitochondria of a character not met with in dealing with other nephrotoxic substances. It was suggested that this unusual alteration was due to the action of residual licheniformin A5 in the renal tissue. As licheniformin itself would theoretically be washed out of the tissues during processing, a fixative consisting of formol saline with 1 per cent (w/v) phosphotungstic acid added was used so that the insoluble phosphotungstate of licheniformin might be preserved during processing. In subsequent batches of animals, part of the kidney reserved for histology was fixed in this special fixative and the remainder fixed in ordinary fixatives—Helly being used as a routine.

Paraffin sections of phosphotungstic formol fixed material were used. A search for some specific method of rendering the supposed licheniformin A5 compound visible led to the trial of numerous sulphonated dyes. Little success was

achieved, as those stains which gave some distinctive colour to the supposed residual deposits also stained the rest of the tissue deeply. It was thought that the phosphotungstate might reinforce the phosphotungstic acid present in Mallory's phosphotungstic acid haematoxylin (phostox). Normally, formalin fixed sections do not take up this stain under 12–24 hours. The phosphotungstic acid formol fixed sections of kidneys showing damage by licheniformin gave an almost immediate and very clear indication of the damaged mitochondria which became a deep blue-black after 10–30 minutes. The only other tissue elements stained in these conditions were elastic and connective tissue fibres. This " phostox reaction " proved of considerable value in detecting early changes and grading the degree of damage.

Formol fixed material from the same animals did not give the " phostox reaction." Kidneys damaged by various other nephrotoxic agents, such as mercuric chloride, uranium nitrate, sodium acid phosphate, and polymyxin A, when processed in a similar way did not give the reaction. The study of series of these animals shows that the " phostox reaction " shows up the earliest and slightest degree of damage; Altmann's acid fuchsin method for mitochondria shows up damage slightly more severe, and the lesions must be very much more advanced to show up when a routine haemalum and eosin stain is used.

The final assessment of damage has been worked out on the histological changes seen in Altmann preparations, as it is possible to compare the changes directly with examples of renal damage by other substances. Also the overall changes in the kidney can be studied in the same preparation as that used for study of the specific mitochondrial change.

An approach can be made to the problem of studying the renal damage either by giving a single subcutaneous dose just below the LD50 level and examining the kidney at two-hourly intervals up to twenty-four hours, or by dosing groups of animals with smaller single doses and examining the kidneys at a fixed period such as twenty-four hours after injection.

By giving a large subcutaneous dose of 10 mg. to groups of animals and examining at two-hourly intervals the following stages of damage have been worked out.

Stage I.—The earliest detectable change is seen at 2 hours. The mitochondria in the cells of the first part of the proximal convoluted tubule show breakdown into granules. The granules pass towards and may be discharged into the lumen with some fragmented portions of cytoplasm. Fraying of the innermost layer of the cytoplasm is found throughout the whole first part of the nephron and can be seen in the straight terminal portion in the subcortical zone.

Stage II.—At 4 hours the mitochondria in the proximal convoluted tubule are almost all broken up, and cytoplasmic fragments with mitochondrial granules are present in the lumen of the first part of the proximal convoluted tubules, and this change extends further down the convoluted part of the tubule. This is most clearly seen in the subcapsular zone of the cortex where the convolutions form the bulk of the renal substance.

Stage III.—At 6 hours the mitochondrial fragments are irregular in size and shape and the lumen is filled with fragments and cytoplasmic debris. The subcapsular convolutions become dilated but are filled with debris. The terminal part of the proximal tubule (the straight tubule) in the deeper layers of the cortex and in the

subcortical zone shows degenerative changes. At this stage the alteration in the renal cells can be recognized in haemalum eosin preparations.

From 6 hours onwards the extent of the changes increases. The maximum alteration in the mitochondria is reached about 15 hours after injection, when recovery or further cellular degeneration occurs, depending on the individual sensitivity of the mice.

Stage IV.—By 24 hours a stage is reached in which fresh cellular damage is negligible. In the first part of the proximal convoluted tubule mitochondrial granules are now large and irregular and may be in the lumen or within the cytoplasm as globular aggregates. In haemalum eosin preparations these aggregates stain with eosin and correspond to the globoid hyaline droplets described by some authors.

At Stage IV the condition of the main mass of the convoluted part of the proximal tubule (best seen in the subcapsular area) is the most characteristic feature. This part of the tubule is dilated and filled with cytoplasmic debris and mitochondrial fragments. The epithelial cells are thinned and their nuclei show changes. In some animals mitoses are frequent, and in others, where the condition seems more severe, the nuclei stain poorly. The lower straight part of the tubule is dilated and contains casts.

Stage V.—At 24 hours after a dose of 10 mg. all changes tend to diminish and restitution takes place in the majority of animals; however, some mice, presumably more constitutionally susceptible, show changes of a more advanced character such as were regularly obtained in the survivors after single doses above the LD50. In mice surviving a dose of 20 mg. at 24 hours the changes of Stage IV are present, but the epithelium of the main mass of the proximal convoluted tubule shows definite necrosis and the lumen of the tubule is filled with cellular debris and casts.

The five stages of tissue-reaction described above can be recognized at characteristic intervals of time not only after a single large dose (e.g., 10 mg.), but the maximal effect of smaller doses can also be described in these terms. Thus single doses of 1, 2, or 5 mg./mouse produce in the kidney within 24 hours, respectively, Stages I, II, and III.

Examination of groups of mice kept for 2–3 weeks after being injected showed that, where only Stages I–III had occurred, no kidney abnormality persisted. Where Stage IV had been reached, slight fibrosis of the kidney insufficient to interfere with renal function might result. In a proportion of mice Stage V is followed by relatively severe changes including glomerular damage.

Table III summarizes the results from some of the experiments on mice in which the reaction to single and multiple doses was studied.

The single 10-mg. dose (nearly LD50) has already been shown to produce a small degree of permanent scarring, and an equivalent single dose of fraction DEA.1 caused slightly less reaction. However, Exps. 4 and 5, in which a comparison was made of the effect of three-hourly doses of the two materials, show that this latter fraction was not less nephrotoxic than the parent material.

Exp. 2 shows that fraction DEA.2, also, was not sufficiently less toxic than the control when single doses of comparable biological activity were given to mice.

TABLE III

THE EFFECT OF SUBCUTANEOUS INJECTIONS IN MICE

Exp.	Dosage		Total units	Blood concn. units/ml. at peak	Kidney concn. units/g. at peak	Kidney histopathology	
	Mg.					Stage	Sequel
1 Batch DB.1 ..	10	Once	60,000	1,300	30,000	IV	Return to normal
Fraction DEA.1	5	Once	54,000	1,100	22,000	II	Return to normal
2 Batch DB.1 ..	2.5	Once	15,000	280	11,000	II	Return to normal
Fraction DEA.2	5.0	Once	15,000	—	6,700	I	Return to normal
3	1.0	Once	6,000	180	6,400	I	Return to normal
4	1.0 × 6	3-hourly one day	36,000	250	18,000	III	
	× 12	Two days	72,000	250	36,000	IV	Fibrosis
	× 18	Three days	108,000	250	21,000	IV, V	Fibrosis and scarring
5 Fraction DEA.1	0.5 × 6	3-hourly one day	32,400		24,000	II	
	× 12	Two days	65,000		36,000	IV	
	× 18	Three days	97,400		37,000	V	Fibrosis and scarring
6	0.25 × 6	3-hourly one day	9,000		9,500	I	
	× 12	Two days	18,000	40	11,000	II	Mild local fibrosis
	× 18	Three days	27,000	50	10,000	III	Slight general fibrosis
7	0.5 × 14	Twice daily one week	42,000	60	9,000	III	Return to normal
	× 28	Two weeks	84,000	70	8,500	III	Return to normal
8	0.2 × 14	Twice daily one week	16,800	40	11,000	II	Return to normal
	× 28	Two weeks	33,600	30	7,500	III	Return to normal

With regard to the toxicity of the bulk material, it will be seen that as the level of dosage is lowered three-hourly doses of 1 mg. (Exp. 4) can safely be given for one day and only a small degree of scarring without impairment of function occurs by the end of the second day. Subsequently, however, further doses cause fibrosis. Reducing the size of the dose to 0.25 mg. but retaining the three-hourly administration results in only temporary changes with, however, a tendency towards early fibrosis in local areas. If the number of doses per day is reduced as in Exps. 7 and 8 from six to two, a larger total amount of licheniformin A5 can safely be given. The longer interval between doses favours excretion, the kidneys suffer less damage, and relatively less drug remains fixed in their tissue; thus doses of 0.5 mg. or 0.2 mg. when given twice daily to mice for two weeks caused only a temporary reaction in the kidneys with return to normal within a short time.

Rabbit, guinea-pig, and rat

The equivalent of three-hourly injections of 1.0 mg. and 0.25 mg. in the mouse was given on a body weight basis to groups of rabbits, guinea-pigs, and rats and continued for two days.

Sensitivity to the nephrotoxic action of licheniformin A5 varied between the species, and the results are summarized in Table IV. The rabbit was easily the least affected, and three-hourly injections at the higher level of dosage produced only minimal kidney reaction, even after two days. The guinea-pig and mouse were about equally sensitive, although it was noted that the guinea-pig survivors

TABLE IV

THE EFFECT OF SUBCUTANEOUS INJECTIONS IN DIFFERENT SPECIES OF ANIMALS

Exp.	Animal species	Dosage Mg./kg.		Total units	Blood concn. units/ml. at peak	Kidney concn. units/mg. at peak	Kidney histopathology Stage	Sequel
9	Rabbit (2,000 g.)	50 × 12	3-hourly 2 days	7,200,000	450	103,000	Almost normal	None
	Guinea-pig (300 g.)	50 × 12	3-hourly 2 days	1,080,000	320	44,000	II	Diffuse fibrosis
	Mouse (20 g.)	50 × 12	3-hourly 2 days	72,000	250	36,000	IV	Fibrosis
	Rat (200 g.)	50 × 12	3-hourly 2 days	720,000	1,900	28,000	V	Diffuse fibrosis, glomerular changes
10	Rabbit (2,000 g.)	12.5 × 12	3-hourly 2 days	1,800,000	140	52,000	Almost normal	None
	Guinea-pig (300 g.)	12.5 × 12	3-hourly 2 days	270,000	60	15,000	I	Irregular slight fibrosis
	Mouse (20 g.)	12.5 × 12	3-hourly 2 days	18,000	40	11,000	II	Mild local fibrosis
	Rat (200 g.)	12.5 × 12	3-hourly 2 days	180,000	270	12,000	IV	Diffuse fibrosis, glomerular changes

when examined three weeks later had more kidney fibrosis than was to be expected from the mild original reaction. The rat, which had already been shown to be more susceptible to the general toxicity of licheniformin, was now found to be very sensitive to the nephrotoxic action.

Three-hourly injections at the lower level of 12.5 mg./kg. confirmed the above order of species sensitivity, the kidneys of the rabbit being scarcely different from normal, the guinea-pig and mouse suffered only to a mild degree, but the rat again showed widespread damage. The rabbit kidney shows remarkably little cellular change considering the relatively high concentration of residual licheniformin A5 per gramme of tissue. On the other hand, the rat kidney shows a marked cellular degeneration although the concentration of drug in the tissue is relatively not high, but it is rather dammed back in the blood stream.

Discussion

Any new antibiotic to be of therapeutic value must prove to be relatively active against one or more species of pathogenic micro-organism, and secondly the effective dose or series of doses must not be toxic to the extent of causing serious permanent damage to the tissues of the host.

Licheniformin has been shown to fulfil the first proviso by the work of Callow *et al.* (1947), who found that the material, *in vitro* and to a certain extent *in vivo*,

significantly inhibited a series of pathogenic micro-organisms including streptococci, staphylococci, the anthrax bacillus, and also, to a certain degree, the tubercle bacillus. A brief *in vitro* study of the inhibitory activity of the licheniformin A5 used in this work has produced similar results with the first three organisms mentioned above. The sensitivity of *M. tuberculosis* was not examined.

Licheniformin A5 is of moderate general toxicity, being only slightly more toxic to mice by the subcutaneous route than is streptomycin. The intravenous route is in general not the safest route for the administration of most of the common antibiotics. However, by this route the LD50 dose of licheniformin A5 for mice is relatively favourable, compared with that of some other antibiotics already in use. Along with some other antibiotics such as polymyxin A, bacitracin, and ayfivin, however, it causes, above a certain low level of dosage, serious damage to the kidneys in certain species of experimental animals.

In order to avoid lasting damage in the kidneys the maximal safe level of subcutaneous dosing in the mouse per kg. body weight was found to be a single dose of 125 mg. Twice-daily doses of 25 mg. could be given for at least two weeks and most probably for much longer, and three-hourly doses of 50 mg. and 12.5 mg. given for one and three days respectively produced no lasting effect.

The resulting blood levels would be a transient concentration of about 250 units/ml. from the single dose, a twice-daily transient peak of about 70 units/ml. from the second course described above, and a maintained level of 250 units/ml. for one day, or 50 units/ml. for three days from the respective courses of three-hourly doses. As the biological unit is based on the *in vitro* inhibition of *M. phlei*, which is approximately 10–30 times more sensitive than *B. anthracis* or *M. tuberculosis*, the blood concentrations resulting from the twice-daily dosing or the three-day course in the mouse—namely, maxima of 70 and 50 units/ml. respectively—would appear to be lower than is desirable as a therapeutic level for combating such pathogens; thus, if a pathogenic micro-organism was only a tenth as sensitive as the test organism *M. phlei* by *in vitro* methods, the above maximal blood concentrations might be considered to represent 7 and 5 inhibitory units/ml. blood for this particular pathogen. It is obvious that these blood levels, which result from two of the finally selected courses of repeated doses in the mouse, have a lower concentration of inhibitory units than is usually aimed at in chemotherapy.

Different species of laboratory animal were shown to differ in their sensitivity to the acute toxic effect of licheniformin A5 and also to its nephrotoxic action; as regards the latter effect the rabbit was relatively unaffected, but the kidney damage suffered by the mouse, guinea-pig, and especially the rat has led to the conclusion that both the wider use of licheniformin A5 in its present state of purity and any extension of animal experiments would be unjustified.

Summary

Some pharmacological evidence has been obtained on the antibiotic licheniformin A5, which is a polypeptide mixture produced by *B. licheniformis* (*A5*) under certain specified growth conditions.

1. The acute toxicity for mice by the subcutaneous route is not great, being 670 mg./kg., which is of the same order as that of several of the other polypeptide

antibiotics, while intravenously (370 mg./kg.) it is relatively less toxic than the others.

2. A proportion of the material injected (initially 10–30 per cent and diminishing as the injections are continued) remains for a time in the tissue of the kidneys, especially in the convoluted tubules of the first part of the nephron. The concentration diminishes subsequently, but after three months 15 per cent of the original level persists.

3. The amounts which can be injected subcutaneously in the mouse without causing permanent damage to the kidneys have been determined by histological study. The maximum daily dosages tolerated per kg. of body weight are as follows (all daily totals): 300 mg., in six three-hourly doses for one day; 125 mg., in a single dose; 75 mg., in six three-hourly doses for three days; 50 mg., in twice-daily doses for 14 days and longer.

4. The mitochondria of the affected cells are prominently involved in the cellular reaction, and the stages in the cellular degeneration are described in detail.

5. Different species of laboratory animals are not equally susceptible to the nephrotoxic action of licheniformin A5. Compared with the mouse, the rabbit is resistant and the rat relatively sensitive.

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References

Arriagada, A., Savage, M. C., Abraham, E. P., Heatley, N. G., and Sharp, A. E. (1949). *Brit. J. exp. Path.*, **30**, 425.

Belton, F. C., Hills, G. M., and Powell, J. F. (1949). *J. gen. Microbiol.*, **3**, 400.

Callow, R. K., Glover, R. E., Hart, P. D'Arcy, and Hills, G. M. (1947). *Brit. J. exp. Path.*, **28**, 418.

Hills, G. M., Belton, F. C., and Blatchley, E. D. (1949). *Brit. J. exp. Path.*, **30**, 427.