PROLONGED INJECTIONS OF MALE SEX HORMONES INTO NORMAL AND SENILE MALE RATS

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An investigation of the effects of prolonged treatment with male hormones of normal—that is, non-castrated—and especially senile, animals has not only theoretical but great practical interest. The majority of patients undergoing treatment with male hormones possess gonads, although usually in an impaired functional condition and often with anatomical changes. Seniles are considered to be especially suitable patients, following the publications of Brown-Séquard (1889a, 1889b), later corroborated in a different way by Steinach's (1912, 1920) experiments on the ligation of the vasa deferentia, and the observations of Voronoff (1923, 1924, 1926) on the effects of testicular grafts.

In spite of the contradictory and often disappointing results of other workers (reviewed by Korenchevsky, 1925; Romeis, 1931a; Sand, 1933) on the effects of these treatments, experimental and even clinical evidence suggests that the internal secretion of the gonads has some significance in the pathology of senility. It is also important to discover whether the sex hormones have any toxic effects, especially as many clinicians are using large doses in their attempts to obtain desired results. Therefore, now that pure sex hormones are available, an investigation into their possible favourable and toxic effects, in particular on senility, is urgently required.

Technique

When interpreting the results obtained in the present investigation it is necessary to take into consideration the following conditions of the experiments. The senile

TABLE I.—The Effect of Male Sex Hormones on Weights of Some Organs of Senile Male Rats

Group :	I	II	III	IV	V
	Rats Injected Weekly with				
	Oil (Control Rats)	Andro- sterone 7.5 mg.	Dehydro- andro- sterone 7.5 mg.	Testost. prop. 0.75 mg.	Testost. prop. 7.5 mg.
Testes (gm.) ..	3.08	2.28	2.63	2.00	2.02
Seminal vesicles (gm.) ..	1.56	1.40	1.43	2.02	3.35
Prostate (gm.) ..	1.80	1.74	1.69	1.85	2.64
Penis (mg.) ..	483	449	407	441	459
Preputial glands (mg.) ..	268	230	208	267	295
Thymus (mg.) ..	187	149	145	117	36
Liver (gm.) ..	16.72	15.84	14.13	11.80	13.66
Abdominal fat (gm.) ..	34	32	27	28	24
Final body weight (gm.) ..	551	477	449	424	454
Gain in body weight (gm.) ..	68	68	2	1	25
No. of rats in group ..	4	4	4	4	4
No. of rats with pneumonia	3	3	2	4	1

rats used did not belong to the same litter, but were taken from the stock at the Institute, and were known to be about 14 to 18 months old at the end of the

experiment. At this age the inbred rats of our stock show definite signs of senility.

Chronic pneumonia occurs in varying degree in nearly all senile rats, and is produced by a germ which is at present unknown. The disease occurred in a mild form in every group of senile rats, as indicated in Table I, but all rats suffering from severe pneumonia and falling in weight have been excluded from the experiments. The difficulty in obtaining large numbers of senile rats, the fact that they come from different unknown litters, and the possibility of some changes due to pneumonia made it necessary for us to corroborate some of our results obtained on old rats by experiments on younger normal rats with an average final age of 137 to 174 days. These rats belonged to known litters, and the litter-mates were so far as possible equally divided among the different groups (Table II).

TABLE II.—The Effect of Male Sex Hormones on Weights of Some Organs of Adult Male Rats (Average Age 138 to 174 Days)

Group :	I	II	III	IV	V	VI	VII
	Control Rats (Oil-injected)		Andro- sterone 7.5 mg.	Testosterone		Testost. prop.	
	Age 165 Days	Age 138 Days		0.75 mg.	7.5 mg.	0.75 mg.	7.5 mg.
Testes (gm.) ..	3.03	2.98	1.64	2.91	2.16	1.67	2.48
Seminal vesicles (gm.) ..	1.41	1.23	0.87	1.55	1.42	1.49	2.96
Prostate (gm.) ..	1.49	1.23	1.11	1.53	1.33	1.67	2.29
Penis (mg.) ..	370	334	302	372	368	395	472
Preputial gl. (mg.) ..	134	95	141	137	111	117	200
Adrenals (mg.) ..	55	52	42	58	55	57	55
Thymus (mg.) ..	293	274	240	258	234	183	108
Liver (gm.) ..	14.63	13.18	11.76	13.74	14.48	12.95	11.81
Abdominal fat (gm.) ..	34	30	32	33	31	25	24
Final body weight (gm.) ..	483	413	390	464	425	413	395
Gain in body weight (gm.) ..	288	358	330	235	196	362	159
No. of rats in group	7	5	4	5	4	3	3
Average age of rats	165	138	144	162	162	137	174

Note.—The data of cols. III and VI have to be compared with those of the control rats of similar age (137 to 144 days) given in col. II; the data in cols. IV, V, and VII (average age 162 to 174 days) with the control data in col. I. The doses given are weekly.

The pure hormones, supplied by Messrs. Ciba Ltd., were dissolved in sesame oil. Five times a week 0.2 c.cm. of the hormonal solution was injected in the doses given in Tables I and II, the control rats receiving pure sesame oil. The organs were fixed in Bouin's solution. They were then weighed and for histological investigation were embedded in paraffin, or frozen sections were cut and stained for lipoid and fat with scharlach red. The sexual organs, hypophysis, adrenals, thyroids, liver, and kidneys have been examined by weight and histologically; the thymus, spleen, and heart by weight only. Other details of our experimental technique were the same as those described in our previous papers (Korenchevsky, 1937; Hall and Korenchevsky, 1938a).

Experimental Observations

The experiments were performed on a total of sixty-three rats; of these, twenty senile (Table I) and thirty-one adult male rats (Table II) were injected for a period of 90 to 118 days. The results were compared with those obtained on twelve senile rats—six controls injected with oil and six rats injected daily with 0.9 mg. of androsterone for twenty-four days only. The latter results are

not given in a separate table as they were similar to those of 118 days' duration. The arrangement of the prolonged experiments is clear from the tables: for economy of space only those organs in which changes occurred have been included. Similarly the weights per unit of body weight have not been tabulated, but, where necessary, are referred to in the text. Since the results obtained on both senile and adult rats were very similar, they will, so far as possible, be discussed together.

External Appearance and Behaviour of the Rats

Senile Rats.—As compared with normal adult rats they have lost the agility, curiosity, and sexual activity of young or adult rats; usually they sit inertly in their cages and are indifferent to their surroundings; their fur is rough and dirty; their skin covered with insects, as they do not clean themselves; and they develop obesity if they do not suffer from a severe form of pneumonia. Because, however, all rats with severe pneumonia were excluded from the experiment, and because of the great care taken in their feeding (Hall and Korenchevsky, 1938a) and keep, our senile rats were not small, extremely emaciated animals, as are those represented in the photographs of some other workers on senile rats. In spite of the features of senility described above, they were large, well-nourished animals with an average weight of about 550 grammes. The condition of their sex organs was also comparatively good, as dissection and histological investigation of the control rats showed. The injections did not noticeably affect the appearance and behaviour of the senile rats: the animals remained inert in their movements and indifferent to their surroundings, and their fur and skin were dirty. In fact, the condition of the senile rats injected with the large dose of testosterone propionate became even worse: they were thinner than the controls and their fur was dirtier. Although only a single case of pneumonia occurred among the senile rats injected with large doses of testosterone propionate (Table I), this may be accidental, and therefore cannot be regarded as definitely indicating a favourable effect of the hormone on this disease.

Adult Rats.—After large doses of testosterone propionate the fur of the adult rats became rough and dirty, and a very marked increase in nervous excitability was also noticed, but nothing abnormal was recorded with smaller doses of this hormone or with the other hormones used.

Sex Organs

EFFECT OF SENILITY

It is remarkable that senility did not produce any signs of degeneration, as judged by weight (Table I, Group I) and histologically, in the sex organs of our rats when compared with those of the normal adult rats (Table II, Group I). Spermatogenesis was well developed in both groups. In his excellent book Romeis records the same in the sex organs of his senile rats, which were well fed and cared for and had no apparent diseases, though the comparatively small weight of his rats and, in some, decreased weight of the testes (Romeis, 1931c) show that the diet was probably still deficient in some respects. The pronounced degenerative changes in the sex organs described by some authors Romeis quite rightly explains by unsuitable diet and complicating diseases. After a critical review of the literature dealing with senile changes in the sex organs of human beings he comes to the conclusion that in the healthy senile man the testes might show no significant changes (Romeis, 1931d) from the normal.

EFFECT OF INJECTIONS ON THE TESTES

All the hormones used caused a decrease in the weight of the testes (Tables I and II); even in the six senile rats injected for only twenty-four days, in which rats they were 2.49 grammes as compared with 3 grammes in the controls. The more pronounced decrease in Groups III and VI (Table II) is explained by the fact that treatment was begun at an earlier age. This also explains why in some rats of Group III (Table II) injected with androsterone there was a complete absence of spermatogenesis, the structure of the testes being that of infantile rats. In all other groups the only histological change observed was a slight decrease in the size of the testicular tubules. Spermatogenesis was present, although in some cases it appeared to be slightly less pronounced than in the control rats.

Similar changes in *short-duration* experiments were obtained after injection of pure male hormones by McEuen, Selye, and Collip (1937), Korenchevsky, Dennison, and Hall (1937), Moore and Price (1937, 1938), Sainton, Simonnet, and Petrescu (1938), and Bottomley and Folley (1938). It is remarkable that the prolongation of the injections up to four months in our experiments with large doses did not produce greater pathological changes. This indicates the absence of a true toxic effect of male hormones on the testes of adult animals.

Since the depressing effect of male hormones on the secretion of gonadotropic hormone by the hypophysis is well established (Moore and Price, 1937; Hertz and Meyer, 1937; Hamilton and Wolfe, 1938; Cutuly and Cutuly, 1938), the changes obtained in the testes may most probably be explained by this decreased activity of the hypophysis. Lately, however, evidence has been obtained that the male hormones, when injected into hypophysectomized rats, produce a better development of generative tissue of testes and stimulation of spermatogenesis instead of a depressing effect (Cutuly, McCullagh, and Cutuly, 1938; Hamilton and Leonard, 1938). Even without hypophysectomy, stimulation of the testes by male hormones can be produced in certain animals—for example, ground squirrels (Wells and Moore, 1936).

EFFECT ON SECONDARY SEX ORGANS

The character of the changes in seminal vesicles, prostate, penis, and preputial glands was similar in each group, being most pronounced in the seminal vesicles. An unexpected and striking result was the fact that the effect of androsterone and dehydroandrosterone (depression of sex organs) was entirely different from that of testosterone propionate (strong stimulation) and even from that of testosterone (apparent absence of activity). This depressing effect of androsterone was present even in senile rats injected for only twenty-four days. The mechanism of these important differences in the activity of the sex hormones on non-castrated rats is explained by the simultaneous influence of two opposite factors:

(1) More or less complete suppression of the natural secretion of sex hormones from the testes, owing to the depression of the hypophysis by the injections and the consequent decreased secretion of gonadotropic hormones. This would lead to atrophy of the secondary sex organs.

(2) This atrophying effect is, however, counterbalanced by direct stimulation of the secondary sex organs by the injected hormones, which, some completely, some less completely, replace the natural testicular secretion. Theoretically one would expect that androsterone and dehydroandrosterone, being the weakest hormones, would be least able to replace this natural secretion; testosterone should give

better results, and testosterone propionate alone (even in castrated rats) could produce over-stimulation of the secondary organs. The actual results obtained (Tables I and II) well corroborate this theory.

The changes in weight and size of the seminal vesicles, coagulating glands, prostate, and preputial glands are explained histologically chiefly by changed amount of secretion in the tubules and ducts of these organs. In only a few rats (those in which the organs were most affected) were there definite changes (correlating with the changes in size of the glands) in the number of the tubules or in the size of the secreting cells.

Effect on Non-sexual Organs

THE HYPOPHYSIS

The hypophyses of all the rats, with the exception of two senile animals, were of normal weight (10 to 14 mg.) and size. The two exceptional rats developed tumour-like hyperplasia of the hypophysis, similar macroscopically to that regularly produced by oestrogens. In one animal, injected with 7.5 mg. of dehydroandrosterone a week, the hypophysis weighed 320 mg., and in the other, injected with 0.75 mg. of testosterone propionate a week, the weight was 144 mg.

We have been able to find only two references to the occurrence in rats of spontaneously developed hypophyseal tumours. Fischer (1926) described tumours (largest 8 by 6 by 6 mm.) in two old female rats and in one male, while Romeis (1931b) mentions merely an enlargement of the hypophysis (the largest weighing only about 45 mg.) in four old rats.

The histological picture in Romeis's rats was adenoma-like growth starting from the anterior lobe, while Fischer's two histologically investigated cases had carcinoma-like tumours with "castration" cells (signet-ring cells with colloid-containing vacuoles) starting from the anterior lobe and in addition, in one case, containing adenoma of the posterior part of the hypophysis. In both of these rats there was also adenoma or adenocarcinoma of the thyroid glands. As malignant features of the tumours Fischer mentions irregular growth (with ingrowth into normal tissue), abundant irregular mitoses, occurrence of gigantic nuclei, and nodule-like formations of tumours sharply differing from the neighbouring normal hypophyseal tissue.

In the light of these observations we cannot therefore exclude the possibility of spontaneously developed tumours in our cases. In support of this is the fact that our tumours had a similar histological structure to those of Fischer.

They consisted of cells varying in size from small to gigantic, containing small or gigantic, sometimes lobulated nuclei, with varying amounts of chromatin. The gigantic cells often contained two or three, seldom more, nuclei. Several mitoses could be seen. The nucleoli were often large. The protoplasm of most of the cells was vacuolated in varying degree, producing in extreme cases "foamy" cells. Some of the nuclei were also vacuolated. As frozen sections were not made, the nature of this vacuolation could not be ascertained, but it was obvious that the vacuoles were of two kinds: (1) similar to the usual colloid vacuolation, present, for instance, in the "castration" hypophysis; and (2) empty vacuoles of dropsical or lipid nature (lipoid having been extracted by fat solvents during embedding). The whole gland was very hyperaemic.

The tumours could be classified as adenomata. Since no invasion into the surrounding tissue and no metastases could be found, the malignant character of the tumours could not be ascertained. The following features suggest, however, that they may be of cancerous nature:

(1) variations in the size and form of the cells and their nuclei; (2) variation in the chromatin content of the nuclei; (3) abundance of mitoses; and (4) presence of giant multinucleated cells. It may be mentioned that in chromophobe adenomata of the hypophyses of human subjects mitotic figures are rare (D. S. Russell, personal communication). There are, however, two points which might suggest that injections of the hormones may at least predispose to, if they do not cause, development of the tumours: (1) in our stock of rats we have never come across spontaneously produced hypophyseal tumours in adult or old rats, although we have dissected more than 6,000 rats, of which at least 100 were senile; and (2) the hyperplasias occurred only in the injected animals, although it must be admitted that the number of animals in each group was small.

Taking all the above into consideration, and admitting the possibility that the tumours may have been of spontaneous origin, one definite conclusion can be drawn—that *male hormones in the doses used do not prevent the development or cause the regression of hypophyseal tumours*. The histological structure of our tumours is entirely different from that of the hypophyseal tumours produced by oestrogens (which are hyperplasias of the chromophobe cells of the anterior lobe). It cannot therefore be probable that in our cases the tumours developed as a result of transformation of male hormones into oestrogens, which transformation is quite possible according to the observations of Steinach and Kun (1937).

ADRENALS

In our previous experiments with *castrated* rats (Hall and Korenchevsky, 1938b) all the male hormones investigated caused a decrease in the average weight of the adrenals. In the present experiments with *normal* animals this effect was not produced except in the case of adult non-senile rats injected with androsterone (Table II, col. III). This is in agreement with our previous experiment of twenty-one days' duration with normal animals injected with testosterone and testosterone propionate (Korenchevsky, Dennison, and Hall, 1937). Histological investigation showed that the lipid content and vacuolation of the cortical cells were decreased, though usually in a much less degree than in our previous experiments on castrated rats, and mostly in the zona reticularis. Testosterone propionate in large doses was the only hormone which caused considerable lipid depletion. The presence of the gonads therefore in most cases prevents the changes in weight of the adrenals and partly prevents the microscopical changes produced by the injected male hormones.

In the adrenal cortex of three senile rats there was occasional dilatation of blood vessels or their sinuses, forming large lacunae. The surrounding cortex cells were enlarged and degenerated. Since, however, one of these rats was an uninjected control and two were injected (one with dehydroandrosterone and the other with testosterone propionate), these dilatations may be considered to have been caused by senility and not by the injections of the hormones. It might be emphasized, however, that the lacunae were very much larger in the two injected rats than in the control, and that these two injected animals were those which developed hypophyseal tumours.

THYMUS AND LIVER

Thymus.—All the hormones hastened the involution of the thymus in the adult rats (Table II) and in the senile rats, both those injected for a long period (Table I) and those injected for twenty-four days.

Liver.—A decrease, insignificant in two groups and definite in the others (Tables I and II), in actual weight of the liver was recorded, even in those senile rats injected for only twenty-four days (14.73 grammes, as compared with 17.14 grammes in the controls). This is in contrast to the hypertrophying effect of male hormones on the livers of castrated rats (Hall and Korenchevsky, 1938a). When, however, the weights are calculated per unit of body weight there is found to be no significant decrease, the changes in liver weight corresponding more or less to the changes in body weight. No histological changes were found in a preliminary investigation.

KIDNEYS

The weights of the kidneys were not changed, except after large doses of testosterone propionate, when the actual weight was increased about 12 per cent. and the weight per unit of body weight 33 to 36 per cent. No pathological changes were observed histologically, but in the kidneys of some of the injected rats, especially after large doses of testosterone propionate, the convoluted tubules were slightly larger or the cells forming them slightly hypertrophied. The changes in weight and histological structure indicate a slightly stimulating effect of the hormones on the kidneys.

Thyroids, Spleen, and Heart.—No changes were observed.

Fat Deposition and Gain in Body Weight

Since the senile rats ceased to grow, and most of them suffered from pneumonia (though in a slight form; see Table I, last line), our conclusions are based chiefly on the data obtained from adult rats (Table II). Androsterone and small doses of testosterone and testosterone propionate (Table II, cols. III, IV, and VI) did not cause any significant changes, while large doses of testosterone, and especially testosterone propionate (cols. V and VII), had a definite depressing effect on gain in body weight. A decreased fat deposition was found after injections of testosterone propionate, especially in large doses. A similar effect on body weight and fat deposition in normal adult rats of large doses of testosterone propionate (but not of testosterone) has been recorded by Korenchevsky, Dennison, and Hall (1937) in experiments of three weeks' duration. In experiments, also of shorter duration, Sainton, Simonnet, and Petrescu (1938) obtained a similar depressing effect of testosterone propionate on body weight, while McEuen, Selye, and Collip (1937) could not produce any definite changes by testosterone injections (2 mg. for 36 to 38 days). The above results suggest that testosterone propionate, and possibly testosterone, influence metabolism.

Toxicity of the Hormones

All the above-mentioned changes produced by the prolonged injections, even of large doses of the hormones, were of comparatively mild nature and (provided that the tumours in the hypophyses of senile rats have not been caused or stimulated by the injections) could not be classified as toxic. The depressing effect of the male hormones on the development of the testes and especially on the secretion of the anterior lobe of the hypophysis should, however, be taken into consideration by clinicians when treating patients.

Summary

CHANGES COMMON TO NORMAL ADULT AND SENILE RATS

The changes observed after injections of androsterone, transdehydroandrosterone, testosterone, and testosterone

propionate in the weight and histological structure of the organs were in almost all cases similar in normal adult and senile rats.

All the hormones depressed in varying degree the development of the testes.

Development of the secondary sex organs was unexpectedly depressed by androsterone and transdehydroandrosterone but not significantly changed by testosterone, and was considerably stimulated by testosterone propionate. An explanation of these differing effects is given.

The size and weight of the hypophyses were not changed except in two injected senile rats, which developed tumours of this gland, different in structure from those produced by oestrogens. Two possible explanations of their origin are given.

Other changes observed in the injected animals were: increased involution of the thymus; decrease in the actual weight of the liver, without histological changes; possible stimulation of the kidneys; some lipid depletion in the cortex of the adrenals; nervous excitability and decreased fat deposition (with testosterone propionate), and (with large doses of testosterone and testosterone propionate) decreased gain in body weight.

SENILE CHANGES

No improvement in the senile appearance and behaviour of the old rats was produced by the hormones in the doses used.

Taking into consideration the fact that our senile rats had normal sex organs, and that these organs and sexual function might be preserved in otherwise senile men, the absence of a rejuvenating effect of the sex hormones is not surprising.

This does not, however, upset the hypothesis that, in combination with other co-operative factors, the sex hormones are important and possibly irreplaceable in the prevention of pathological senility.

The stimulation of the secondary sex organs of senile rats by testosterone propionate cannot be regarded as a rejuvenating effect, since, without simultaneous improvement of the general condition and of the other features of senility, this artificial sex stimulation is biologically unnatural and in patients is medically undesirable.

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THE SULPHANILAMIDE TREATMENT OF GUINEA-PIGS INFECTED WITH BRUCELLA ABORTUS

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During the past few months numerous reports have appeared in this country and the United States of America of cases of undulant fever apparently successfully treated with prontosil or sulphanilamide. In other but less numerous reports no benefit is recorded. In the laboratory Chinn (1938) found that sulphanilamide had a bactericidal effect *in vitro* on *Brucella abortus*, while Britton (1938) failed to observe any such effect. Montgomerie (1938) inoculated guinea-pigs with *Br. abortus*, and about four weeks later began treatment with sulphanilamide. He gave 100 to 200 mg. daily over a period of twelve days and killed the animals ten days later. In three out of four of his treated animals the tissues were culturally sterile. Chinn (1938), who gave 100 mg. daily for a month, starting treatment two hours after inoculation and killing his animals at the conclusion of the course, was even more successful, since he was unable to demonstrate the presence of infection in any of his treated group of six animals. The experiments recorded in this paper were begun before these laboratory reports were published, though Mr. Montgomerie kindly showed us an advance copy of his paper.

Experiment 1

On March 4, 1938, ten male and ten female guinea-pigs, weighing 300 to 400 grammes, were inoculated intramuscularly into the left thigh with 800,000 living organisms

Group B.—Starting on March 14, four animals received 100 mg. daily for ten days; two were killed a day after and two a week after the conclusion of treatment.

Group C.—Starting on March 21, four animals received 150 mg. daily in two divided doses for ten days; two were killed a day after and two a week after the conclusion of treatment.

The sulphanilamide was given by mouth suspended in gum tragacanth. None of the animals received treatment on Sundays. One or two control animals were killed at the same time as each subgroup of two infected animals. Since these subgroups overlapped, the result was that there were always three, and sometimes four, control animals for comparison with each infected group.

In making the bacteriological examination the animal was anaesthetized with ether, the thorax opened, and blood taken for agglutination from the inferior vena cava. The great vessels were then cut and the animal was allowed to bleed to death. The spleen and sublumbar glands were excised, ground up separately in Griffith tubes and suspended in N/4 Ringer solution; viable bacterial counts were then made on the suspensions by the roll-tube method. In addition the macroscopic appearance of the glands, spleen, and liver was carefully noted, particular attention being paid to the degree of enlargement of the organ as a whole and to the number and size of any focal lesions that might be present. The results were recorded in a series of index numbers varying from 0 to 4. The summarized results are recorded in Table I. (The figures in both tables represent arithmetic mean values, with the exception of agglutinin titres, for which the geometric mean has been used. The figures in the columns headed "Macroscopic" refer to index numbers, with a maximum of 4.)

On the whole the treated animals showed fewer macroscopic lesions, had lower agglutinin titres, and were less heavily infected than the control animals. But the results were disappointing. In only two of the treated animals were the sublumbar glands sterile, and in not one of the treated animals was the spleen sterile. Judging from the number of colonies developing from the sublumbar glands and spleen the animals in Group C did better than those in Group B, and those in Group B better than those in Group A; but the same held true, though to a less extent, of the control animals, showing that the disease was beginning to undergo natural retrogression. It is clear that under the conditions of this experiment the effect of sulphanilamide fell far short of complete sterilization of the tissues.

TABLE I.—Experiment 1: Comparison of Treated and Control Guinea-pigs

Group	No. of Animals	Treated							Control						
		Macroscopic			Colonies		Agglut. Titre			Macroscopic			Colonies		Agglut. Titre
		Subl.	Spl.	Liver	Subl.	Spl.				Subl.	Spl.	Liver	Subl.	Spl.	
A (100 mg. for 5 days) ..	4	0.5	0.5	0.5	12,000	32,000	0		3	1.0	0.3	2.7	16,000	37,000	1/50
B (100 mg. for 10 days) ..	4	0.8	1.0	0.8	1,600	2,400	1/3		4	1.3	1.0	2.5	6,300	36,000	1/270
C (150 mg. for 10 days) ..	4	0.8	0.5	0.8	50	720	1/10		3	1.3	1.3	2.3	1,000	30,000	1/40

Subl. = Sublumbar glands. Spl. = Spleen.

washed off from a two-day liver-agar culture of a virulent CO₂-sensitive strain of *Br. abortus* (Astor). Six animals were kept as controls. The others were treated as follows:

Group A.—Starting on March 7, four animals received 100 mg. of sulphanilamide daily by the mouth for five days; two were killed a week after and two a fortnight after the conclusion of treatment.

Experiment 2

On July 29, 1938, twelve male and twelve female guinea-pigs, mostly weighing 400 to 600 grammes, were inoculated intramuscularly into the left thigh with 600,000 living organisms washed off from a two-day liver-agar culture of *Br. abortus* (Astor). Of these animals twelve were kept as controls; the remainder were treated as follows: