THE INFLUENCE OF THE ADRENAL GLANDS ON RESISTANCE.

I. The Susceptibility of Adrenalectomized Rats to Morphine.

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One of the early theories in regard to the function of the adrenal gland was that it acted as a detoxifying agent (1, *a*). While no convincing proof of this hypothesis was presented, many scattered facts have been brought out which show that the adrenals are involved in the reaction of the organism to infection and intoxication, and which suggest that these glands play an important rôle in the protective mechanism of the organism. In severe infections and intoxications pathological changes in the glands occur regularly, varying in intensity from congestion and edema to hemorrhages and foci of necrosis (2, 3) and the characteristic lipoids in the cortex show striking alterations in distribution and amount (4, 5). Chronic infections and intoxications in animals are accompanied by a marked hypertrophy of the cortex (6, 7). These effects of acute and chronic infections and intoxications on the adrenals invite the consideration of the influence of these glands on the resistance to such conditions.

In addition to the pathological changes mentioned, the early literature dealing with this relationship of the adrenal confines itself chiefly to three lines of argument: (1, *b*) the resemblance of the effects of adrenal ablation to a progressive intoxication (1); (2) the toxic properties of blood and tissue extracts from adrenalectomized animals, and conversely the protective properties of normal blood in adrenalectomized animals (8, 9, 10); and (3) the protection afforded by mixtures of the adrenal gland or its extracts with various toxic agents (11, 12).

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None of these points is supported by convincing evidence of a detoxifying function of the adrenal. Entirely non-specific changes explain most of the phenomena under the third heading (13).

The direct approach to the problem by determining the resistance of surviving adrenalectomized animals has been attempted by several investigators. Most of the earlier reports were unsatisfactory due to the lack of sufficient control (unsatisfactory methods, no operative control animals, etc.) In 1921, however, Lewis (14) studied the resistance of adrenalectomized rats to several drugs in a comprehensive and adequately controlled manner. After reviewing the previous results with this method, he reported a definite increase in the susceptibility of rats after adrenalectomy to a number of different drugs. Morphine gave the most notable result, in that $\frac{1}{400}$ of the minimal lethal dose was sufficient to kill adrenalectomized rats (15). Stewart and Rogoff were unable to confirm these results (16).

This type of reaction to adrenal insufficiency deserves careful study on account of its importance in throwing light on the functions of the adrenal glands, and especially in regard to the involvement of the adrenals in infections. It is, therefore, the purpose of this paper to report the effect of adrenalectomy on the susceptibility of rats to morphine. This is undertaken as a preliminary to the investigation of the influence of the adrenal glands on the resistance to infections and intoxications.

The adrenal gland in mammalia is an organ composed of two separate tissues, cortex and medulla, entirely distinct in origin, morphology, and cellular chemistry. No definite correlation in the function of these two tissues has been proven. In certain lower species they exist as separate organs (1, *c*). The variations in the resistance of adrenalectomized rats to morphine observed in this study are believed to be entirely dependent on the removal of cortical tissue, and not related to epinephrine. This point will be discussed later.

Methods.

Experimental Animal.—The choice of the appropriate experimental animal is a very important matter in all work with the adrenals on account of the significant variations in these glands in the different species. The best method so far reported for producing an adrenal insufficiency giving symptoms, without causing death, is by adrenalectomy in a series of animals possessing adrenal accessories (17). The rat and rabbit are the only common adult laboratory animals of which a large proportion possess cortical accessory tissue. On this account a large number of these two species will survive double adrenalectomy, a fact amply attested by the confusion which it caused for many years in demonstrating that adrenal tissue is essential to vertebrate life. The survival of adrenalectomized rats was not explained until it was shown that this animal usually possessed numerous accessory cortical rests far removed from the adrenal gland, chiefly in the vicinity of the sex glands, and microscopic in size (18). For reasons of economy of materials and work the rat is much to be preferred in such a study, where a relatively large number of animals are required. Stock rats of the Bussey Institute, *Mus norvegicus*, predominantly white with some color admixture, were used.

Procedure.—Rats under constant and identical laboratory conditions were observed for several days. Then at the same session six to twelve rats were operated upon alternately by one of two procedures, of which the first was double adrenalectomy, and the second was some control operation producing similar traumatization. This usually consisted in single adrenalectomy with some manipulation on the opposite side which left that adrenal and its blood supply intact. 7 days later, those rats that were fully recovered, eating well, and indistinguishable in behavior from normal rats, were injected with morphine sulfate.

It became evident early in this work that certain factors, not absolutely necessary with normal animals, were essential in the handling of adrenalectomized rats. All animals were kept in separate cages before and after operation, the room temperature was kept constant at 75–78°F., and they were on a completely adequate diet for several days before operation and continuing throughout the experiment. The diet used was kindly furnished us by Dr. Reid Hunt and was of the following formula.

Rolled oats	15 parts
Hominy	60 "
Dried meat scrap	14 "
Dried milk powder	10 "
Salt	1 "

15 gm. of this mixture were given the rat each day, and that remaining from the day before was weighed to calculate the amount eaten. The individual cages had two compartments, one containing the food-dish and water-tube, the other, paper shaving bedding. Water was constantly available.

A very good judgment as to the condition of the rat can be formed by an estimate based upon the following four factors: (1) food intake, (2) weight variation, (3) character of stool, and (4) behavior. The measurement of food intake before operation is valuable as determining the individual base line. Normally the rats eat 10 to 15 gm. per day, though a reduced amount for any one day is without significance.

Diminished appetite is one of the most important signs of illness in rats. Any change in weight taken in conjunction with the food intake offers a clue as to the metabolic condition of the animal. On the diet specified the stools are invariably firm in the controls, while diarrhea develops in some of the adrenalectomized rats. As to behavior, the animals used were all active, invariably reacted definitely to blowing (though to quite different degrees), and kept their eyes open after being aroused; while the deviation from this behavior in the experimental animals varied from inactivity and loss of appetite to coma. A very striking picture was occasionally presented as a most severe reaction, either after operation or injection; *i.e.*, an adrenalectomized rat losing weight rapidly, with a persistent diarrhea, eating little or nothing, sitting very quietly in a hunched-up position, fur ruffled, eyes nearly closed, and not responding at all to gentle blowing.

Operation.—Adrenalectomy in rats has been repeatedly performed by previous investigators. The lumbar or back route with incisions through the abdominal wall on both sides has been employed (19, 20). It was found at the beginning of this work that a single midline ventral incision was as satisfactory and more expeditious for bilateral adrenalectomy. Ether anesthesia was always used, by the open method after induction. The operative field was prepared by shaving and bichloride sponges. The incision, 2 cm. in length, begins just below the xiphoid. After opening the abdomen a nasal speculum is carried between the lower lobe of the liver and the right kidney, and on opening the blades the right adrenal is exposed as a pinkish yellow body 1½ to 4 mm. in diameter, embedded in a small fat strip running along the vena cava. With a double ring-curret-bladed biting forceps the edge of which has been dulled the adrenal is now picked up. (The adrenal slips into the opening of the rings and is not crushed.) A small curved clamp is put on the pedicle, and by traction on the forceps, while the clamp is held rigid, the adrenal is pulled from its bed. Bleeding may be controlled by a ligature or clip around the pedicle which is caught by the clamp, but usually a sponge tip carried to this point and held with gentle pressure will control all bleeding after removing the clamp. The capsule is usually intact. Care must be exercised in putting the clamp on the pedicle not to catch the vena cava or renal vein, and any traction on this forceps is apt to tear the vena cava. Usually the right adrenal in the rat is far enough from the vena cava to be removed in this way without much difficulty. This anatomical relationship is another important advantage of using rats rather than rabbits, since the vena cava wall has frequently to be partially ligated in removing the rabbit's right adrenal.

The removal of the left adrenal of the rat is even more simple than the right. The spleen is caught by its tip and delivered out of the wound. With the nasal speculum opened between the left kidney and the mesenteric attachment of the spleen the left adrenal is exposed, embedded in a rather moveable mass of fat.

It is removed in the same manner as the right, the spleen returned to the abdomen, and the wound closed in two layers with silk. At no time are the intestines deviscerated. The whole operation usually requires only 10 minutes; within another 15 minutes the animal is walking about its cage.

That shock does not play an important rôle in the outcome is shown by an analysis of the first 100 rats operated upon in this way, 58 of which had double adrenalectomy, and 42 single adrenalectomy. Only three of these animals died within 3 days; two of the doubly and one of the singly adrenalectomized, and in each of these cases a severe hemorrhage was responsible. In two of the first fifteen adrenalectomies, the capsule was broken at one point and a small piece of it left. The cortical cells rapidly undergo compensatory hypertrophy under such circumstances. In none of the later cases has a fragment of the gland been found at autopsy.

Presentation of Data.

Results of Adrenalectomy in Rats.

On the basis of the results of adrenalectomy in the first fifteen rats, the precautions previously mentioned as being important in the care of such animals were evolved. This original group underwent approximately the same operative procedures as later groups, but they were kept before and after operation in stock cages each containing several rats, and on a varying diet consisting chiefly of oats with bread, milk, and cooked meat scrap. Under this regime they were in excellent condition before operation, yet of eleven doubly adrenalectomized rats seven died in from 3 to 9 days; four survived and seemed in good health at the end of a week. The four singly adrenalectomized survived without symptoms.

The standard balanced diet and individual cages were then introduced and in the next 46 rats, 25 were doubly adrenalectomized with only three deaths. This contrast is shown graphically in Table I. Whether the unbalanced diet or the segregation is responsible for the heavy mortality in the first group has not been worked out. However, that it is due to a metabolic disorder is suggested by the development of a severe diarrhea which persists to death although the animal is eating almost nothing and is put into a separate cage. The protocol of Rat 13 illustrates this type of reaction very well. In seven of the ten deaths of adrenal insufficiency definite hemorrhagic necrotic areas were found in the mucous membrane of the stomach.

At the end of a week after adrenalectomy, if the rat is active, eating well, and has not lost 10 per cent of its body weight, it will survive indefinitely under optimum conditions. But a certain number of such animals are still sensitive to conditions that they would have survived before operation. They may die if put back into stock cages.

TABLE I.

Mortality of Double Adrenalectomy in Rats.

	Total.	Survived.	Died.	Mortality.
				per cent
Stock group	11	4	7	64
Rats in individual cages on standard diet	25	22	3	12

Susceptibility to Morphine.

1. In Operative Controls.—Seventeen rats were used as operative controls. One adrenal was removed and some operative procedure carried out on the opposite side, so that the factors of the operation would be as nearly as possible identical with double adrenalectomy. The right adrenal was usually excised, as this is technically more difficult, and is occasionally accompanied by hemorrhage. On the left side the procedure usually consisted in the excision of a piece of fat in the immediate vicinity of the adrenal gland; sometimes the capsule was stripped from the upper pole of the kidney. If a hemorrhage was encountered in excising the first adrenal, that rat was used as a control animal and the second adrenal was not removed. A small dose of morphine was purposely chosen in order that minor variations in susceptibility dependent on such factors as hemorrhage, appetite, etc., would not appear in the results. Of these seventeen operative controls fourteen survived doses ranging from 50 to 100 mg. per kg., and three died, a mortality of 18 per cent. (Table II shows the exact doses given.) The fatal cases were interesting; one was pregnant, the second had some obscure pathological condition with periportal changes in the liver and hemorrhages into the remaining adrenal, and no reason for the fatal termination in the third was apparent.

2. In Doubly Adrenalectomized Animals.—Twenty-two doubly adrenalectomized rats were injected with morphine sulfate subcutaneously in doses ranging from 10 to 30 mg. per kg., the maximum being $\frac{3}{5}$ of the minimum dose given the control rats. Fifteen of these rats were killed by such small amounts of morphine; seven survived, the mortality being 68 per cent (Table III). This effect of adrenal insufficiency is shown by a comparison of the different results of small doses in the adrenalectomized and control animals. Five of eight adrenalectomized rats were killed by $\frac{1}{10}$ of the minimum dose that was fatal to any of the operative control animals.

The three adrenalectomized rats that had survived 10 mg. per kg. were reinjected 11 days later (18 days after operation) with 30 mg.

TABLE II.

Resistance of Operative Controls.

Morphine sulfate, *mg. per kg.*	100	80	60	50	Totals.
Total No.	9	2	2	4	17
Survived	6	2	2	4	14
Died	3	0	0	0	3

TABLE III.

Resistance of Adrenalectomized Rats.

Morphine sulfate, *mg. per kg.*	30	20	10	Totals.
Total No.	7	7	8	22
Survived	3	1	3	7
Died	4	6	5	15

per kg. of morphine sulfate. Two of the three died, and one survived. As a matter of fact, then, of the twenty-two adrenalectomized rats tested, seventeen (77 per cent) were killed by morphine sulfate in amounts of 30 mg. per kg. or less. Only the results of the original injection are entered in Table III. Both adrenalectomized and control animals were usually injected 1 week after adrenalectomy; a few were tested in the 2nd week.

Only those adrenalectomized rats were used for this purpose that were active and eating well at the time of injection. However, it was apparent that many of the adrenalectomized animals did not eat well for 2 or 3 days after operation, while the controls usually had regained a normal appetite within 24 hours. This fact was reflected in the average weight losses after operation. In the control rats it was 1 per cent, while in the group of adrenalectomized rats it was 4.6 per cent at the time of injection. Furthermore, the seven adrenalectomized rats that survived morphine lost only 2.7 per cent in weight, while the fifteen that were killed by morphine had an average weight loss of 5.3 per cent. This comparison suggested that the difference in food intake might be a factor in the decreased resistance. But this hypothesis seemed unlikely, as a few of the susceptible adrenalectomized rats ate well even immediately after operation, and an occasional one of the control animals had a poor appetite and lost weight, yet survived the larger doses of morphine. The lack of correlation between food intake and susceptibility of individual rats is illustrated by Protocols 2 and 3.

3. In Controls with Diminished Food Intake.—The effect of a diminished food intake was tested in the following manner. Operative control rats were prepared as in the previous experiments. They received no food for 48 hours after operation. The first group of three were then each given 2 gm. of food a day. 7 days after operation they had thus received a total of 10 gm. of food and had lost 28 per cent (average) of their pre-operative weight. They were given 50 mg. of morphine per kg. Two of the three survived this dose and one was killed by it. It was obvious from the weight loss and food intake that this was a much more drastic diminution in nutrition than any of the adrenalectomized rats had experienced.

Therefore, in the next group of four rats, they were fasted for 2 days after operation and then allowed 4 gm. of food apiece per day. On this ration the weight loss in each case was greater than the maximum (11 per cent) found in any of the adrenalectomized rats. 1 week after operation these four controls, whose weight loss averaged 15 per cent, were injected subcutaneously with 60 mg. of morphine sulfate per kg. (twice the maximum dose given any adrenalectomized animal). All survived this dose. Thus, of the seven animals with greatly diminished food intake, only one was killed by morphine, though given in much larger doses than to any of the adrenalectomized rats. It seems justifiable, then, to conclude that the decreased resistance of adrenalectomized rats is not due to a diminished food intake. (Table IV.)

4. By Intravenous Administration.—In order to eliminate any variation in absorption, four rats were injected intravenously with morphine sulfate solution. Two operative controls were given 45 mg. per kg., which was about $\frac{1}{2}$ of the minimum lethal dose for unoperated rats. Before the injection had been completed respiration had become imperceptible, and the rats were rigid when put back into their cages. Within a few minutes respiration became perceptible; the condition of the rats improved gradually. In 3 hours the coma had disappeared, and the rats were making voluntary movements, though they were still quiet. The following day they had completely recovered, were alert, active, and had eaten their full rations (15 gm.).

Two adrenalectomized rats were injected at the same time, one with 9, the other with $4\frac{1}{2}$ mg. per kg. ($\frac{1}{5}$ and $\frac{1}{10}$, respectively, of the dose given the operative controls). The immediate effect of these small doses was in sharp contrast to the complete prostration and respiratory paralysis of the control animals. Both rats became quiet, but did not stop breathing, and they made voluntary movements on stimulation. Instead, however, of showing evidence of recovery within 3 hours as the controls had done, their condition became worse, and both died.

TABLE IV.

Resistance of Operative Controls with Diminished Food Intake.

	No. of rats.	Food intake per rat, 1 week.	Weight loss (average).	Morphine sulfate.	Survived.	Died.
		gm.	*per cent*	*mg. per kg.*		
	3	10	28	50	2	1
	4	20	15	60	4	0
Total.	7	10 to 20	15 to 28	50 to 60	6	1

From one of the operative controls that survived the morphine intravenously, the remaining adrenal was removed after an interval. A week after the second operation, being in excellent condition, it was injected intravenously with $\frac{1}{2}$ of the dose that it had formerly withstood. The immediate effect was less pronounced than previously, but the rat became progressively worse and died 36 hours later. In this one animal, then, is seen survival, with one adrenal intact, following a dose double that which proved fatal to it after the second adrenal had been excised (Protocol 4). The same striking increase in susceptibility to morphine is demonstrated in adrenalectomized rats when the drug is administered intravenously.

TABLE V.

Comparative Resistance of Rats after Excision of One Adrenal and of the Second.

Rat No.	Weight at operation.	Adrenal excised.	Days between operation and injection.	Weight at injection.	Injection of morphine sulfate.	Result.	Remarks.
	gm.			*gm.*	*mg. per kg.*		
10	140	Right.*	7	144	100	Survived.	14 days between operations.
	145	Left.	6	152	10	"	Fragment of right adrenal not
			15	180	20	"	removed.
			25	175	100	"	
16	340	Right.	11	320	100	"	18 days between operations.
	327	Left.	7	320	10	"	
			10	305	50	Died.	
17	340	Right.	11	335	100	Survived.	18 " " "
	332	Left.	7	325	10	"	
			10	315	50	Died.	
18	250	Right.*	11	260	100	Survived.	18 " " "
	?	Left.	7	260	10	"	Fragment of right adrenal not
			10	252	50	"	removed.
19	320	Right.	10	305	100	"	17 days between operations.
	307	Left.	7	278	20	Died.	
22	402	Left.	10	379	100	Survived.	17 " " "
	362	Right.	7	367	30	Died.	
53	202	Right.	7	147*	50	Survived.	13 days between operations; only
	180	Left.	7	185	30	"	10 gm. of food given in first week after first operation.
54	199	Right.	7	148*	50	"	13 days between operations; only
	175	Left.	7	175	30	Died.	10 gm. of food given in first week after first operation.

* See under remarks.

5. In the Same Rats After Single and Double Adrenalectomy.—From eight operative control rats the second adrenal was removed and they were reinjected subcutaneously with smaller amounts of morphine. Six of them had survived 100 mg., and two 50 mg. per kg. of morphine sulfate after one adrenal had been removed. After the second adrenal had been removed, five of the eight were killed by 50 mg. per kg. or less. Time intervals, weights, and exact doses of morphine are given in Table V. It is interesting to note that two of the three rats that survived had a fragment of adrenal left *in situ* at the first operation, as subsequently discovered.

Subacute Type of Reaction to Morphine.

In normal animals morphine given subcutaneously produces its effect within a short time, terminating in death or recovery. The outcome is decided within 24 hours, usually in much less time than this. Our operative control animals were alert, active and eating well on the day following the injection in almost every case. Morphine sulfate injected subcutaneously or intravenously was fatal to twenty-six rats doubly adrenalectomized in 1 or 2 stages. Twelve of these (46 per cent) died more than 24 hours after injection. The most striking instance of this reaction ocurred in Rat 24; though profoundly affected by the drug, it lived for 10 days after injection (Protocol 5). Others lived for 3 to 5 days. They never recovered their appetite or normal behavior. Some of the doubly adrenalectomized rats that survived their small doses of morphine were below par for 2 days after injection, while the control animals receiving two to ten times as much morphine were invariably normal in behavior 24 hours afterwards.

DISCUSSION.

In opposition to Stewart and Rogoff (16), the statement of Lewis (15) that rats are much more susceptible to morphine after adrenalectomy, has been confirmed in this study. The cause of the discrepancy seems apparent from Stewart and Rogoff's protocols. None of their rats was tested within a period of 6 weeks after operation whereas a marked hypertrophy of adrenal cortical cells will occur within 4 weeks. This fact is illustrated by one of our rats in which a piece of adrenal cortex, too small to be seen, was left at operation. 26 days later the hypertrophied fragment was found to be $\frac{2}{3}$ the size of the original gland. Wiesel (18) has shown that the adrenal accessory rests of cortical cells occurring in the neighborhood of the sex glands undergo compensatory hypertrophy.

The cause, it appears, of the increased susceptibility to morphine during a phase of adrenal insufficiency is not a local traumatization of structures in the neighborhood of the adrenal as our control operations and those of Lewis seem conclusively to show. It is also not due to a general, non-specific disturbance of the endocrine balance since Olds (21) found no increased susceptibility in thyroidectomized rats to morphine. It would seem that a decreased resistance to morphine is a definite effect of adrenal insufficiency, either directly or through some secondary reaction.

Hunt and Seidell (22) have demonstrated that feeding thyroid extract to rats increases as much as ten times their susceptibility to morphine. This suggests that the increased susceptibility of adrenalectomized rats might be due to a stimulation of thyroid secretion.

Marine and Baumann (23) have shown that thyroid function is necessary for the characteristic increased heat production of adrenal insufficiency in rabbits. Scott (24) has presented some evidence of a rapid depletion of thyroid colloid by partial adrenal insufficiency in cats. Both of these reports present indirect evidence of an autogenous increase in the thyroid hormone in certain types of adrenal insufficiency. However, there is an important difference in the reactions of adrenalectomized and of thyroid-fed rats to morphine.

In Hunt's study, 51 thyroid-fed rats were given about the minimum lethal dose (23 survived and 28 died); also 12 normal rats were reported (7 survived and 5 died). The susceptibility of the rats receiving thyroid was increased in varying degrees. In every instance, though, the result was determined in less than 24 hours. In the thirty-three fatal cases, all but two of the rats died within 12 hours (these lived 13 and 20 hours, respectively). Contrast with this the effect of morphine observed in adrenalectomized rats. Susceptibility is increased, but one-half of the fatalities occur from the 2nd to the 10th day after injection. In view of this dissimilarity, it seems improbable that increased thyroid function is chiefly responsible for the diminished resistance of adrenalectomized rats.

No attempt was made to determine the minimum dose that could be found to kill the most susceptible adrenalectomized rats. A relatively small number of the adrenalectomized rats are extremely sensitive. Substances have proven fatal to such that a majority of adrenalectomized animals survive. (This is probably due to the marked variation in the amount of cortical accessory tissue.) Therefore, the minimum lethal dose seems to be a less satisfactory method of comparison with adrenalectomized rats.

It was considered far more important for the purposes of this investigation to determine the type of reaction in a number of adrenalectomized rats. They were, therefore, given the drug in doses that were never fatal to the operative control animals. By this method one of the most instructive phenomena observed was quite unexpectedly discovered; namely, the fundamental disturbance of the adrenalectomized rat's condition lasting several days following the morphine injection, and usually terminating in the death of the animal (though evidence of it was also discernible in some of those that recovered). This type of reaction has not been emphasized by previous investigations, and is easily overlooked when the M.L.D. is used as the sole basis of comparison. It occurred only in adrenalectomized animals, and was never seen following single adrenalectomy, even in a partially fasting animal. This profound and unusual effect of morphine on the condition of adrenalectomized animals seems to us to be of great theoretical importance.

The decreased resistance in adrenal insufficiency could be caused by (1) failure to excrete or destroy the drug, and (2) increased susceptibility to a given concentration of the drug.

1. Failure to Rid the Body of the Drug.—Marshall and Davis (25) have shown that in the total adrenal deficiency of cats, even while the blood pressure is still maintained, the nitrogen waste products are excreted much more slowly, and accumulate in the blood. It is not known whether this is the case in the relative insufficiency of adrenalectomized rats (cortical accessories present). However, only a small proportion of the morphine is excreted by the kidneys, and nothing is known about the efficiency of the intestinal excretory function or about the rate of destruction of drugs in adrenal insufficiency. That the persistence of the drug is responsible seems unlikely, from the long period (10 days) that such animals may survive, and from the relatively small amount of the drug given.

2. Increased Susceptibility to a Given Concentration.—The conclusion that there is an increased susceptibility of the animal to a given concentration of the drug seems to be forced upon us. The mechanism of this is not clear. The subacute form of reaction to morphine in the adrenalectomized animal strikingly resembles the cases of fatal adrenal insufficiency seen in rats. Lack of appetite, marked loss of weight and reactivity, terminal diarrhea, and hemorrhages into the gastric mucosa are usual in each of these conditions, though if the morphine-injected adrenalectomized rats had not been given the drug they would have lived indefinitely without symptoms. The similarity is sufficiently marked to suggest that both conditions are fundamentally related.

Final proof as to the nature of the mechanism whereby adrenal insufficiency affects the susceptibility of an animal is not available. But the most logical explanation of the known facts seems to us to be as follows: Insufficiency of adrenal function greatly diminishes some normal metabolic reserve factor. If this effect is too great the animal dies of adrenal insufficiency. Under optimum conditions, though, most rats show little abnormality. However, the effect of a harmful substance such as morphine is sufficient to overcome the diminished reserve factor. The subacute effect is seen when the reserve is used up by the reaction but not greatly exceeded. On this hypothesis the variation in the effect of a given amount of morphine is explained by the various degrees of adrenal insufficiency due to the different amounts of cortical accessory tissue in each rat. Lewis also explains the diminished resistance to certain drugs as a metabolic disturbance, which increases the sensitiveness of cells to toxic substances, and suggests that the mechanism of this may be a quantitative or qualitative change in their lipoid equilibrium (14).

No new evidence is here presented as to which part of the adrenal gland is essential in maintaining the resistance to morphine. The variations in resistance seem to be correlated with the status of the accessory adrenal rests which are made up of cortical cells. It seems improbable that the secretion of adrenin plays any part in the diminished resistance following adrenalectomy. However, further evidence on this point will be presented in a later paper.

The effect of diet in adrenal insufficiency is an important problem which should be carefully studied. McCarrison (26) has pointed out marked pathological changes in the adrenal glands due to unbalanced diets. A completely balanced, uniform diet was given our rats after the first few experiments. The results of these first fifteen adrenalectomies, however, suggest that animals in a state of partial adrenal insufficiency are susceptible to dietary factors that would produce no symptoms in normal animals.

The high mortality of adrenalectomy reported for rats in much of the older literature is undoubtedly due chiefly to the operative technique. Thus H. and A. Cristiani (27) report only three rats out of twenty-nine surviving adrenalectomy for more than 20 hours; and Schwarz (19) claims that removal of both adrenals at one operation is fatal. However, in rats the mortality of adrenalectomy may be high in spite of a perfectly adequate operative technique. Stewart and Rogoff (28) report eight fatalities in 1 to 14 days out of a group of thirteen rats. This corresponds closely with our results in the first fifteen adrenalectomies. By establishing (1) a carefully controlled standard diet, (2) isolation, and (3) uniform room temperature, our mortality immediately dropped from 64 to 12 per cent while the technique of operation was unchanged.

This study was undertaken to determine the types of reaction of adrenal-insufficient animals to the harmful effects of a drug. It is intended as an introduction to the investigation of the influence of adrenal function in bacterial intoxications.

CONCLUSIONS.

1. The majority of rats (*Mus norvegicus*), because of accessory cortical tissue, will survive double adrenalectomy indefinitely under optimum conditions.

2. Resistance to morphine is greatly diminished in healthy adrenalectomized rats tested before hypertrophy of the accessories occurs.

3. This greater sensitiveness seems to be due to some fundamental alteration in metabolism dependent on a partial adrenal insufficiency.

Protocol 1.—Rat 13; brown and white, male. Sept. 15, 1922. In stock. Sept. 18. Active, vigorous, and very vicious. Weight 281 gm. Operation, double adrenalectomy. Prompt recovery. Sept. 21. Very active and vicious. Sept. 22. Jumps viciously this morning on opening the cage. This afternoon, quiet and marked diarrhea. Transferred to individual cage. Eating nothing. Sept. 23. Diarrhea. Quiet. Sept. 24. Diarrhea diminished, more active, but is not vicious. Appetite poor, ate 5 gm. Sept. 25. Diarrhea, ate nothing. Sept. 26. Diarrhea still marked, ate nothing. Died this afternoon.

Autopsy.—Weight 215 gm. Thyroid, heart, and lungs normal. Abdomen: intra-abdominal fat almost completely gone. Adrenals absent. Stomach: definite multiple minute hemorrhages in the mucous membrane throughout the pyloric part. Intestines: several loops of small gut deep red in color. Liver: nutmeg color.

Protocol 2.—Rat 43; white, male. Oct. 20, Active. Weight, 236 gm. Operation, excision of right adrenal and excision of piece of pancreas near left adrenal.

Moderate bleeding. Oct. 22. Has not eaten since operation. Oct. 23. Active, beginning to eat. Oct. 27. Active, eating well now. Slight diarrhea. Weight, 222 gm. At 11:10 a.m. injected subcutaneously with 1.78 cc. of 1 per cent morphine sulfate solution (= 80 mg. per kg.). 1:30 p.m., quiet. 6 p.m., recovering. Oct. 28. Active, eating well. Nov. 10. In good condition. Sacrificed Jan. 8. Right adrenal absent, left adrenal intact.

Protocol 3.—Rat 42; white, male. Oct. 20, Active. Weight, 320 gm. Operation, excision of both adrenals, much abdominal fat. Oct. 21. Ate 10 gm. of food. Oct. 22. Active, ate all of food (15 gm.). Oct. 27. Active. Weight, 310 gm. At 11 a.m. injected subcutaneously 0.62 cc. of 1 per cent morphine sulfate solution (= 20 mg. per kg.). 1:30 p.m., marked pilomotor effect. Did not move. 6 p.m., very quiet. Oct. 28. Very quiet, not eating. Oct. 29. Quiet. Ate a little today. Oct. 30. Very dull, not eating. 5 p.m., comatose. 9:30 p.m., found dead.

Autopsy.—Weight 277 gm. Diarrhea. Thyroid large and dark red. Heart and lungs normal. Abdomen: scant fat. Both adrenals absent. No accessories seen. Right testicle congested. Liver and spleen normal. Stomach: one hemorrhage beneath mucosa near fundus.

Protocol 4.—Rat 21; white and brown, male. Sept. 27. Active. Weight 345 gm. Operation, excision of left adrenal, easy operation. Oct. 4. Active, eating well. Weight 333 gm. At 10:48 a.m. injected 0.75 cc. of 2 per cent morphine sulfate intravenously (= 45 mg. per kg.). 10:50 a.m. respirations imperceptible. Board-like rigidity. 11:10 a.m. respirations very shallow. Complete coma. 1 p.m. condition gradually improving. Oct. 5. Alert and active, eating well. Oct. 11. Active, eating well (15 gm. per day). Weight 335 gm. Operation, excision of right adrenal. Easy operation, no hemorrhage. Moderate fat. Oct. 18. Excellent condition, active, good appetite. Weight 321 gm. At 3:50 p.m. injected 0.74 cc. of 1 per cent morphine sulfate intravenously (= 23 mg. per kg.). 3:52 p.m. respirations imperceptible for a minute, and rat is stiff. 3:57 p.m. voluntary movement. Oct. 19. 9 a.m. very quiet. Marked ruffling of fur. Not eating. 6 p.m. very quiet and dopey. Oct. 20. Found dead this morning.

Autopsy.—Well preserved fat. Diarrhea. Thyroid rather large and red. Heart and lungs normal. Abdomen: both adrenals absent. No accessories seen. Stomach: five distinct hemorrhages into mucous membrane, one is 3 x 4 mm.

Protocol 5.—Rat 24; white and gray, male. Sept. 27. Active. Weight 320 gm. Operation: double adrenalectomy. Easy operation. Sept. 29. Eating well, alert. Oct. 2. Active, ate 12 gm. Weight 318 gm. Oct. 3. Ate 10 gm. Oct. 4. Active. Weight 311 gm. Ate 12 gm. At 10:30 a.m. injected 0.28 cc. of 1 per cent morphine sulfate intravenously (= 9 mg. per kg.). Became quiet but did not stop breathing. 5 p.m. quiet and fur ruffled. Oct. 5, very quiet. Eyes closed most of time. Hunched up, fur ruffled. Drinks with eyes closed. Not eating. Oct. 6. Head between front paws most of time. Pilomotor reaction marked. Oct. 7. Condition about the same. Has eaten nothing since operation. Oct. 8. Seems slightly more active, not eating. Oct. 9. Ate 5 gm. of food, first since injection. Oct. 10. Very drowsy. Stools firm and white. Not eating. Oct. 14. Very weak. Has not eaten 10 gm. since injection. Oct. 15. Found dead this morning.

Autopsy.—Weight 240 gm. Thyroid moderate size. Heart in systole. Lungs normal. Abdomen: adrenals absent. No accessories seen. Left testis converted into a sack filled with dark red grumous material. Right testis atrophied. Stomach: postmortem change.

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