behaviour of their Cβ substances ; and that the Cα substances determine the serological specificity of the various smooth types.

SUMMARY.

1. The smooth cholera vibrio possesses four serologically active polysaccharide or non-protein carbohydrate-containing constituents—here termed Cα, Cβ, Cγ and Cδ. Of these, Cα, the determinant of smooth-type specificity, is lost in roughening, exposing Cβ, the characteristic rough polysaccharide. On degradation to the ρ form Cβ disappears, but Cγ and Cδ—substances common in whole or in part to many vibrios—remain.

2. On digestion of cholera vibrios with papain in a slightly acid medium Cα and Cγ are brought into solution ; Cβ and Cδ are not, but may be liberated from the residue with alkali.

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THYMUS AND ADRENALS IN THE RESPONSE OF THE ORGANISM TO INJURIES AND INTOXICATIONS.

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IN conditions of intoxication by drugs, some organs react more readily than others. The thymus, the adrenal and the liver seem to be particularly sensitive. Diseases or experimentally-produced lesions of the adrenal or of the liver decrease the resistance of the organism to numerous toxic substances. These findings made it very likely that the thymus, the adrenal and the liver play an important role in the defence reaction of the organism against drugs and traumatic injuries. In the present communication experiments are described which were designed to clarify some of the inter-relations which exist between these three organs after traumatic injuries and during various intoxications.*

* A short summary was presented at the Washington Meeting of the Federation of the American Societies for Experimental Biology, March, 1936.

EFFECT OF FASTING, VARIOUS OPERATIVE INJURIES AND DRUGS.

The fact that various drugs, infectious and other diseases may cause a sudden so-called " accidental " involution of the thymus has long been known, but it has also been established that quantitatively and qualitatively inadequate nutrition will produce similar results. Both malnutrition and intoxication will also cause adrenal enlargement and decrease in liver size. It seemed important, therefore, to establish what part malnutrition plays in the production of the changes occurring in these three organs during various defence reactions of the organism, for it is obvous that any severe injury will decrease the appetite of the animal and thus cause malnutrition.

The normal variations in size of these organs are considerable, and are greatly influenced by age, sex, diet and various environmental conditions. I used, therefore, inbred female rats of the same colony, 2–5 months old, fed on a uniform " Purina " diet ; but even within this age-range one can only compare groups born in the same month, for drugs will lead to much more rapid thymus involution in 5-months-old animals than in 2-months-old ones. Owing to the great individual variations, particularly in thymus size, we had to draw our conclusions from averages of large experimental series, so that over 1500 rats were necessary to complete this work. It is impossible to report on each individual experiment without prolonging this paper unduly, and only representative, sample experiments, necessary to substantiate our conclusions, will be discussed.

In order to establish the effect of fasting on the weight of thymus, adrenal and liver in the rat, four groups of normal females were fasted for 1, 2, 3 and 4 days respectively. The weights of the organs in question at the end of these periods are reported in Table I, together with normal controls. These data show that no considerable decrease in thymus size occurs until the end of the second day, and that on the other hand animals fasted for 4 days or longer invariably show pronounced thymus involution. I concluded, therefore, that any experimental condition which will produce pronounced thymus involution (that is, an average thymus weight below 100 mg.) within 48 hours acts in itself, and not simply by decreasing the appetite. It is evident, furthermore, that since 4 days' fasting will invariably produce severe thymus atrophy, any experimental condition which will maintain the thymus weight at its normal level in an animal fasted for this length of time must have an inhibiting effect on thymus involution.

After a few preliminary experiments with various drugs, we found that atropine, morphine and formaldehyde are particularly active in producing rapid thymus involution. As may be seen from Table II, atropine in doses of 2 c.c. of a 1 p.c. solution twice daily subcutaneously, or morphine in doses of 2 c.c. of a 1 p.c. solution twice daily subcutaneously, or formaldehyde in doses of 0·5 c.c. of a 4 p.c. solution twice daily subcutaneously, invariably produce severe thymus involution within 48 hours after the first injection. Since 48 hours' fasting has no marked effect on thymus size, we fasted our experimental animals during the injection period, so as to avoid variations due to differences in the amount of food consumed. From the same table it may also be seen that the adrenals enlarge quite rapidly, and the increase in

TABLE I.

Normals.	Thymus weight (mg.).						Adrenal weight (mg.).						Body weight (g.).					
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
2 months old. Fed. . .	187	318	340	325	295	321	31	34	42	36	30	26	112	138	163	162	136	125
3 „ „ „ . .	190	315	241	298	300	340	56	44	68	50	46	36	159	164	183	162	156	138
5 „ „ „ . .	171	150	152	304	205	160	52	48	38	56	32	56	182	161	156	190	168	174
2½ „ „ Fasted 24 hrs. .	193	174	254	213	293	235	34	26	34	39	37	39	109	106	113	170	120	113
3 „ „ „ 24 „ .	170	195	161	248	183	212	39	52	42	53	41	46	157	166	170	140	132	163
2 „ „ „ 48 „ .	151	203	228	161	190	220	44	45	70	51	51	46	134	130	180	140	127	150
3 „ „ „ 48 „ .	150	135	141	104	140	203	44	40	41	34	39	60	150	208	154	138	155	178
5 „ „ „ 48 „ .	146	121	71	61	120	105	44	50	49	51	48	40	176	157	190	155	159	160
3 „ „ „ 72 „ .	113	20	92	95	58	132	50	48	54	38	50	40	139	120	160	130	120	145
3 „ „ „ 96 „ .	67	70	115	77	60	90	54	60	58	62	49	50	145	164	153	148	120	170

TABLE II.

Normals.	Thymus weight (mg.).						Adrenal weight (mg.).						Body weight (g.).					
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
Atropine sulphate: 2 c.c. of 1 p.c. solution twice daily for 48 hours. 3 months old .	37	58	35	38	74	90	54	70	66	71	75	48	136	147	122	145	160	155
Morphine sulphate: 2 c.c. of 1 p.c. solution twice daily for 48 hours. 3 months old .	88	85	60	70	105	62	40	46	50	40	60	60	124	171	105	115	135	158
Formaldehyde: 0·5 c.c. of 4 p.c. solution twice daily for 48 hours. 3 months old .	77	85	78	20	65	58	60	58	58	70	58	57	168	210	144	197	153	156
Adrenaline (1/1000): 0·2 c.c. twice daily for 48 hours. 3 months old .	123	73	23	112	107	67	78	40	58	42	40	45	173	120	112	150	141	113
Adrenaline (1/1000): 0·1 c.c. twice daily for 48 hours. 5 months old .	72	60	95	85	80	86	60	58	64	56	60	50	170	193	183	161	171	172

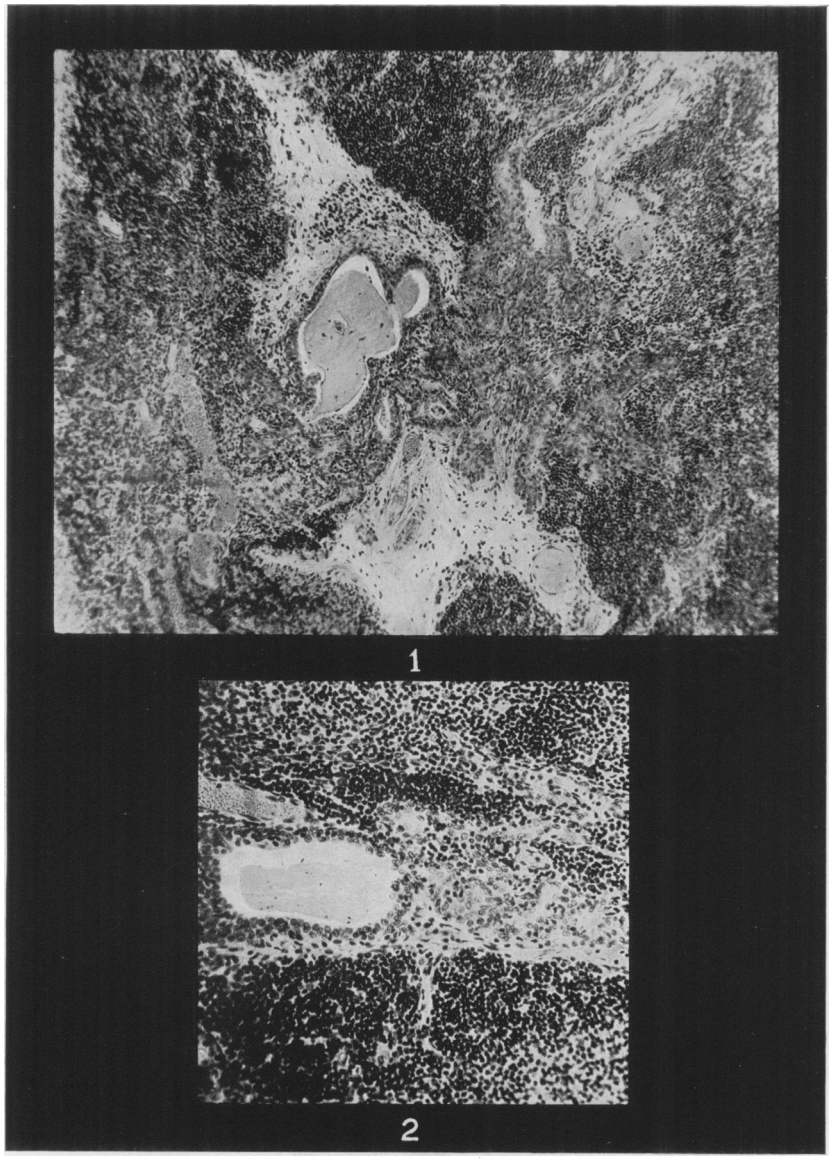

FIG. 1.—Thymus of a rat 48 hours after initiation of morphine treatment. Intrathymic epithelial structure: on the left vesicles filled with colloid resembling thyroid tissue; on the right massive strands of polygonal epithelial cells of "parathyroid-like" appearance.

FIG. 2.—Thymus of a rat treated in a manner similar to that in Fig. 1, showing again typical vesicle formation on the left and massive epithelial strands on the right.

their size is very closely proportionate to the decrease in thymus size. The lipoid content of the adrenals is decreased during this acute hypertrophy, so that their colour is brown and not yellow as it is normally. This loss of lipoids is a symptom of increased activity, according to various authors. It is of interest to note that definite changes in the thymus are already detectable as early as 6 hours, and sometimes even sooner, after the administration of these drugs. The first reaction is a considerable œdema of the organ, which causes an apparent increase in its size during the first hours following the injection. At this time some fluid is usually found in the pleural cavity, and a more generalized tendency to œdema formation manifests itself by the occasional appearance of retroperitoneal œdema.

Histological examination of the thymus after injection of these drugs shows: 24 hours after involution begins the thymocytes degenerate and the chromatin of their nuclei disintegrates into free basophilic granules, which are the most characteristic feature of this first stage of involution. The interlobular connective tissue is œdematous, and degenerative changes appear also in the blood-vessels of the organ.* The lymph-glands of the thymus are usually enlarged, and their sinuses are filled with large wandering cells containing nuclear debris of thymocytes and red blood-cells. Hæmorrhages into the thymus tissue are also frequently observed. After 48 hours the œdema usually subsides and the nuclear debris begins to disappear from the gland. At this stage and later tubular epithelial structures appear quite frequently in the thymus. They may contain eosinophilic colloid and resemble thyroid tissue, or they may be solid. In the latter case their resemblance to parathyroid tissue is so striking that one wonders whether the so-called accessory parathyroids found in the thymus might not be identical with these bodies. The photograph of an intrathymic accessory parathyroid in Spreter's (1935) publication, for instance, is certainly very similar to the epithelial structures we see in involuting thymuses (see Figs. 1 and 2). In a normal, well-developed thymus we have not seen such structures. Nothing can as yet be said about their function, but their glandular structure in the absence of an excretory duct makes them very similar to endocrine tissue. In cases of very advanced involution, almost the entire gland consists of extremely atrophic cells, with very small and intensely basophilic nuclei. This "basophilic" atrophy may occasionally be seen in small areas of normal thymuses, but in these it is always limited to little patches. This patchy basophilic atrophy is not uncommon in other glands such as the thyroid and the parathyroid.

The marked enlargement of the adrenals, and the fact that adrenaline secretion has frequently been found to be increased after administration of toxic substances, made us wonder whether the thymus involution is not simply due to hyperadrenalinæmia. In order to test this hypothesis, we had to establish first whether adrenaline would have the same effect as the other drugs used. The data in Table II show that adrenaline in doses of 0·2 c.c. of a 1/1000 solution twice daily subcutaneously will cause just as severe and rapid thymus involution as the other drugs previously used. But this finding

* We believe that this change is identical with the "Crise caryoclasique" of Dustin (1921), but we do not feel that it represents a specific reaction to certain drugs with "actions caryoclasiques", since we observed the same changes after surgical injuries even though no drugs were injected.

in itself is of course no proof that adrenaline plays any role in the causation of thymus involution by other drugs.

Since we have frequently seen in the course of other experimental series that the thymus of animals autopsied soon after a major operation is usually very small, we wished to establish whether this post-operative involution is simply due to decrease in appetite, or to some more specific influence of the surgical shock on the thymus. Table III shows the effect of various operative injuries on the thymus of animals fasted during the 48 hours following operation. From these data we see that extensive skin-lesions (the skin has been detached from the subcutis over large areas), bone fractures (tibia and femur were fractured under anæsthesia on both sides), peritoneal injuries (all the intestines were placed outside the peritoneal cavity for one minute), and even the mere excitement of an animal the free motion of which is interfered with (by tying the legs together or wrapping the animal tightly in a towel), will cause more or less pronounced thymus involution. At the same time the adrenals are enlarged and usually free from lipoids.

A group of 12 rats kept at a low temperature varying from +1 to 7° C. showed much more marked thymus involution and adrenal enlargement after 48 hours than their fasting controls. The effect of high temperature (35–40° C.) on the thymus was less marked, however. As an incidental observation, it may be of interest to mention that with one exception all the animals kept at a low temperature of +1 to 3° C. developed gastric ulcers. This was also the case in another series of 8 rats kept at a temperature of —2° C. during 24 hours. It is tempting to suspect some correlation between the so-called "hyper-adrenalinæmia of excitement", causing the well-known hyperglycæmia of excitement, and the adrenal enlargement and thymus involution of our experiments.

EFFECT OF ADRENALECTOMY ON THYMUS INVOLUTION.

It seems evident from our tables that the drugs and operative procedures which produce thymus involution almost invariably lead to hypertrophy of the adrenals, and the size of these two glands is approximately in inverse proportion. It appeared to be of interest, therefore, to investigate the possible *role* that the adrenals may play in the process of thymus involution. For this purpose a study of the effect of some injuries and drugs on the thymus of adrenalectomized rats was made. Naturally only such drugs and injuries were used which were found to produce thymus involution in experiments on normal animals. Table IV summarizes the results of this series.

It appears that, in the absence of the adrenals, none of the drugs and injuries which would lead to thymus involution in normal animals will have any effect on the thymus. A secretion of the adrenal gland must therefore be considered essential for the ability of the thymus to undergo sudden involution.

Some indications of a thymo-adrenal inter-relationship have already been pointed out in the literature. Thus it has repeatedly been stated that the thymus enlarges during adrenal insufficiency. Star (1895) was probably the first to call attention to the fact that thymus hypertrophy occurs in Addison's

TABLE III.

Injuries.	Thymus weight (mg.).						Adrenal weight (mg.).						Body weight (g.).					
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
Skin lesion: 48 hours. 3 months old. Fasted	83	151	80	128	78	102	48	45	41	50	49	38	165	136	155	190	155	138
Bone lesion: 48 hours. 3 months old. Fasted	103	203	148	169	92	153	44	46	54	48	57	48	132	153	144	128	147	151
Legs tied: 48 hrs. 3 months old. Fasted	30	90	96	71	75	131	79	74	40	78	44	94	192	197	140	170	171	209
Legs tied: 48 hrs. 5 months old. Fasted	84	95	82	105	65	118	44	60	50	58	41	60	159	196	189	160	171	161
Wrapped in towel: 48 hours. 5 months old. Fasted	120	25	80	114	67	72	60	61	64	54	56	51	197	190	160	170	159	159
Exposed to cold, 1–3° C.: 48 hrs. 5 months old. Fasted	56	82	35	40	48	41	56	70	52	48	50	50	152	151	179	156	152	179
Exposed to heat, 35–40° C. 48 hrs. 5 months old	106	99	58	104	131	81	52	50	42	48	40	39	203	189	173	169	165	151
Peritoneal lesion: 48 hrs. 3 months old	145	79	85	115	80	95	50	72	52	74	82	69	185	153	167	135	193	152
Medulla extirpated: fasted 96 hrs. 5 months old	180	72	81	101	185	156	—	—	—	—	—	—	154	153	143	147	153	167
Exposed to cold, 5–7° C. 48 hrs. 3 months old. Fasted	115	130	75	125	89	77	68	69	59	71	67	54	149	181	142	149	137	152

TABLE IV.

Adrenalectomized: On water.	Thymus weight (mg.).						Body weight (g.).					
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
Legs tied: 3 months old. Died within 36 hrs.	267	167	287	231	190	163	153	134	129	139	172	143
Formaldehyde: 0·5 c.c. of 4 p.c. solution twice daily. 3 months old. All died within 48 hrs.	254	156	228	210	183	171	168	132	150	145	163	145
Adrenaline (1/1000): 0·2 c.c. twice daily. 3 months old. All except Nos. 1 and 4 died within 48 hrs.	199	178	196	198	270	183	133	140	143	130	144	157
Controls: adrenalectomized. 3 months old	200	226	244	218	248	198	150	134	130	144	132	145
2 months old. Fasted 48 hrs.	208	275	248	188	231	175	126	159	134	132	137	132
2½ months old. Fasted 96 hrs.	241	196	225	240	225	230	110	95	120	110	115	174

disease, and this was later confirmed by other authors. Boinet (1899) reported that thymus enlargement may be elicited by adrenalectomy, and the same has been found since by many others. We find no data in the literature concerning the effect of adrenalectomy on the ability of the thymus to undergo accidental involution, except a statement made by Jaffe (1924) that adrenalectomized rats dying within 5–6 days after operation have thymuses 39 p.c. below normal in size, and this is attributed to the fact that "suprarenalectomized animals succumbing gradually in the course of some days do not take food and consequently thymus involution due to inanition and intoxication manifests itself". Another relevant statement is made by Marine *et al.* (1924), who state that thyroidectomy not only prevents the hyperplasia of the thymus in the adrenalectomized rabbit, but actually causes involution in spite of the absence of adrenal tissue. They conclude: "The thyroid hormone is therefore necessary for thymus hyperplasia which usually follows sufficient but sublethal injury of the suprarenal function." A careful study of the papers referred to above and of our own experimental findings makes it very doubtful whether thymus hyperplasia in the strict sense of the word ever occurs after adrenalectomy. Let us take, for instance, the very exact study of Jaffe, which we are in a better position to discuss than that of others, since it was done on the rat, and gives an accurate description of the experimental conditions. From his data it is evident that the thymus of suprarenalectomized rats killed at the age of 208 to 212 days is much larger than that of normal controls of the same age, but its weight is not higher than that of normal rats at the age of 30 to 90 days. Our experiments seem to indicate that the action which the adrenal exerts on the thymus is fully explained by the assumption that it makes the sudden so-called accidental involution of the latter possible. In the absence of the adrenal gland, all those stimuli which cause thymus involution in normal animals are inert. It seems quite likely, therefore, that the effect of adrenalectomy in the hands of previous investigators was only to restore the thymus to a size which equals (or almost equals) the size which the organ usually has at the time of its full development. In normal animals, age, and all the incidental damaging influences which so easily affect the size of the thymus, cause it to involute more and more with time; but all these stimuli act through the adrenals, and are inactive in their absence, so that the thymus will be much larger after adrenalectomy than that of normal animals of the same age.

The question arose which of the adrenal hormones is responsible for the effect which this gland has on the thymus. Since adrenaline secretion is known to be induced by various stimuli which lead to excitement, and a great number of drugs, we first thought that this might be the hormone in question. This suspicion was further corroborated by the finding reported above—that adrenaline itself is extremely active in causing thymus involution. In order to test this theory, one would have to show that adrenaline will cause thymus involution in the adrenalectomized animal. From Tables IV, V and VI we see, however, that this is not the case. Adrenalectomized animals are extremely sensitive to adrenaline, and most of them die during such an experiment, but even those which live long enough to be significant show no thymus involution. From this we would conclude that it is not adrenaline which causes thymus involution in the normal animal. As this is one of the most important points

TABLE V.

Adrenalectomized : Saline given 24 hours before and during experiment.	Thymus weight (mg.).						Body weight (g.).					
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
2½ months old. Fasted 96 hrs.	282	241	162	185	262	140	95	131	100	100	95	100
Legs tied : 3 months old. All lived 46 hrs.	135	212	115	142	187	180	159	139	189	145	145	130
Formaldehyde : 0·5 c.c. of 4 p.c. solution. 48 hrs. 3 months old	175	225	175	168	230	139	140	123	127	147	170	133
Controls : adrenalectomized. 3 months old ; given food and saline 48 hrs.	277	190	241	130	277	222	141	134	138	137	120	144
Adrenaline (1/1000) : 0·2 c.c. twice daily 48 hrs. 3 months old	60	242	293	231	244	260	100	180	145	220	173	217
Formaldehyde : 0·5 c.c. of 4 p.c. solution 96 hrs. 3 months old	290	325	176	115	194	225	142	144	120	123	115	119
Adrenaline (1/1000) : 0·2 c.c. twice daily 96 hrs. 3 months old	220	240	205	237	167	310	138	135	118	120	140	143

TABLE VI.

Adrenalectomy + adrenaline + cortin.	Thymus weight (mg.).						Body weight (g.).					
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
Adrenaline (1/1000) 0·2 c.c. twice daily + cortin 1 c.c. per day, 48 hrs. 2½ months old. One died after 3 injections	183	173	213	140	188	199	134	144	147	128	148	130
Adrenaline (1/1000) : 0·2 c.c. twice daily + cortin 0·5 c.c. per day, 48 hrs. 2 months old. One died. Saline given	198	181	224	210	200	275	139	142	122	148	141	131
Cortin : 4 c.c. per day. 48 hrs. 2 months old	146	225	170	—	—	—	128	111	137	—	—	—
Adrenaline (1/1000) : 0·2 c.c. twice daily + cortin 4 c.c. per day, 48 hrs. 2 months old	205	242	145	148	—	—	116	138	127	135	—	—

for the interpretation of our experiments we wished to obtain more evidence for it.

Treatment with sodium chloride is known to improve the general condition of adrenalectomized rats considerably, so that their body-weight and even their sexual cycles will be maintained. From Table V we see, however, that even though salt-treated adrenalectomized animals maintain their normal resistance to toxic doses of various drugs, their thymuses will be unable to involute after fasting, toxic doses of drugs, and various surgical injuries. The salt was administered in the form of an 0·9 p.c. NaCl solution, which was given instead of drinking-water. With the help of salt treatment, it was possible to maintain adrenalectomized rats treated with 0·2 c.c. of a 1/1000 solution of adrenaline twice daily subcutaneously over long periods, and we found that even 4 days' treatment with this hormone caused no thymus involution. Since even 48 hours' treatment would have decreased the weight of the thymus considerably, in the presence of the adrenals, we cannot consider adrenaline as the adrenal hormone acting on the thymus.

The experiments with salt-treated rats, on the other hand, show that the resistance to drugs is almost completely restored to normal. Numerous experiments with various drugs such as morphine, atropine and adrenaline—not all of which are included in our table—showed that even doses high enough to kill a considerable percentage of the normal animals will not kill a much higher percentage of adrenalectomized, salt-treated rats. This seems to be of interest particularly because it is known that adrenalectomy has a specific effect on the resistance of animals to toxic substances, and the restoration of their resistance has repeatedly been used as a means of assaying cortical hormone preparations (Perla and Marmorston-Gottesman, 1931, and Leloir and Novelli, 1933). Gaunt and co-workers (1934) think that the development of accessory adrenal tissue plays a great part in helping animals to survive for a long time when treated with salt. Since our experiments were performed on the second day after adrenalectomy, development of accessory tissue could not yet have taken place. It may be of some help in explaining the mechanism of salt action to note that the great efficiency of sodium chloride on the restoration of drug resistance was observed only in fed animals; fasted adrenalectomized animals were just as susceptible to drugs whether they received salt treatment or not. We should like to mention in this connection that similar findings have been made in cases of Addison's disease, in which salt treatment proved to be efficient only if the patient took sufficient nourishment (Browne, personal communication).

After adrenaline and salt treatment proved ineffective in restoring the ability of the thymus to involute, it was necessary to establish whether cortin would have this effect. *A priori*, it would have been possible that either cortin or a combination of cortin and adrenaline would be effective. In order to test this possibility, we gave 0·2 c.c. of adrenaline (1/1000) subcutaneously twice daily for 4 days to a group of adrenalectomized rats kept on salt treatment and injected subcutaneously with 5–10 Swingle and Pfiffner dog-units of cortin daily,* the total amount being divided into 4 doses. Such protracted

* We are greatly indebted to the Connaught Laboratories in Toronto for the cortical extract supplied for these experiments through the courtesy of Prof. C. H. Best.

administration increases the activity of a given dose of cortin greatly, as shown by Kutz (personal communication), in the rat. The amount of adrenaline given in this experiment would have produced thymus involution within 48 hours in normal animals, as our experiments show; in these adrenalectomized animals, however, no thymus involution was seen after 4 days of treatment, even though cortin and salt were given. These doses of cortin were large anough to restore the adrenalectomized animal's resistance to adrenaline, but apparently had no effect on the thymus. In order to see whether still larger doses of cortin would have any effect, 8 c.c. of the cortical extract containing 10 dog-units per c.c. were injected in divided doses within 48 hours to 4 adrenalectomized, 2 months old, female rats receiving 0·2 mg.

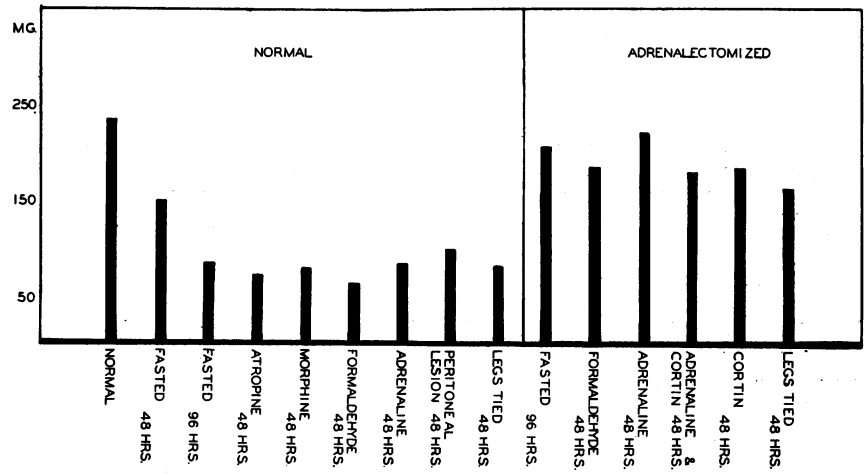

FIG. 3.—The black areas indicate the weight of the thymus under the various experimental conditions, and have been obtained from averages of large groups of rats. It would appear that the thymus of untreated animals is even larger than that of adrenalectomized, treated ones; but owing to the great individual variations it is questionable whether this is significant. It is obvious, at any rate, that those stimuli which produce thymus involution in normals are not active in the absence of the adrenals.

of adrenaline twice daily at the same time, and to 3 others also adrenalectomized but not treated with adrenaline. Even these enormous doses of cortin were not able to cause a thymus involution of the same order as that obtained in the non-adrenalectomized rat. The average weight of the thymus, however, was slightly below that of the untreated controls; but considering the unusually large quantity of extract administered in this series, it is hardly possible to decide whether this slight decrease in weight is due to cortin itself or to traces of a contaminating substance in the extract. The results of these experiments are summarized in Table VI.

In order to see whether the medulla of the adrenal is essential for the action of this gland on the thymus, we removed the adrenal medulla in 6 rats. After making a small incision through the cortex, the borders of the wound were everted by pressure on the opposite pole of the gland; this

exposed the medulla in a very satisfactory way. With the help of a very thin capillary glass tube connected with a suction pump, we proceeded to remove the soft and brittle medullary tissue by suction. This allowed of removal of the medulla without seriously injuring the cortex. In fact histological examination of such glands showed that the cortical tissue was well vascularized and alive, while the medullary cavity was filled with necrotic tissue only. If such animals are fasted for 4 days and then autopsied on the 5th day no marked thymus involution occurs, which seems to show that the medulla is essential for the action of the adrenal on the thymus (see Table III). These findings would seem to indicate that the adrenals play a part in the accidental involution of the thymus which is not wholly explicable in terms of secretion of either or both of the known adrenal hormones, adrenaline and cortin. However, one has to consider the possibility that the amount of adrenaline and cortin necessary to produce thymus involution is even larger than the enormous doses which we used. In this case one would have to assume that more than 80 dog-units of cortin are produced by the adrenal of the rat within 48 hours, under the influence of such conditions as have been found capable of producing thymus involution during this time. The results of the part of our experiments thus far discussed are summarized in Fig. 3.

EFFECT OF HYPOPHYSECTOMY ON THYMUS INVOLUTION.

Since the adrenal is known to be largely dependent upon pituitary function, we were interested to see whether hypophysectomy would have any effect on the ability of the thymus to involute. The publications concerning the effect of hypophysectomy on the thymus are rather contradictory. There are no data in the literature concerning the ability of the thymus to undergo involution under the influence of external stimuli.

In a series of experiments on rats hypophysectomized 8 days before the experiment was started, we studied the effects of such damaging influences as would produce thymus involution in normal animals. Table VII summarizes our findings. We see that hypophysectomy produces only very slight thymus involution, if any, in the rat, and that the thymus of hypophysectomized animals does not undergo as marked accidental involution as that of normals. It seems, however, that a certain degree of involution may occur in spite of the absence of the hypophysis. It is possible that the sudden breakdown of the adrenals after hypophysectomy would liberate such hormones from the gland as are necessary for the production of thymus involution. This might explain the rather contradictory findings reported in the literature. Another possibility to be considered is that a short period of increased activity may follow hypophysectomy in glands standing under pituitary control. At any rate our experiments show that the removal of the hypophysis in itself has little effect on the size of the thymus, but a certain degree of thymus involution can occur immediately after hypophysectomy, under the influence of external stimuli. It seems that the secretion by the adrenal of the hormone causing thymus involution is inhibited, but not completely abolished, in the absence of the hypophysis. Since it is only the cortex which shows definite atrophy

TABLE VII.

Hypophysectomized.	Thymus weight (mg.).						Adrenal weight (mg.).						Body weight (g.).					
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
24 hrs. after hypophysectomy : 2 months old	207	245	201	303	246	353	37	39	25	43	24	30	126	114	110	106	128	140
Legs tied : 3 months old. All died within 24 hrs.	198	218	180	140	230	148	30	42	32	32	45	33	149	159	139	159	129	136
Formaldehyde : 0·5 c.c. of 4 p.c. solution. 3 months old. All died within 24 hrs.	121	150	132	200	121	130	40	35	36	40	36	36	118	140	118	142	133	152
Adrenaline (1/1000) : 0·2 c.c. twice daily. 3 months old. All died within 24 hrs.	107	215	105	154	130	187	40	44	41	50	39	46	119	145	145	150	139	137
Fasted 96 hrs. 5 months old	101	119	94	104	90	87	39	37	41	40	37	39	175	186	175	178	169	132

TABLE VIII.

Hypophysectomized + saline.	Thymus weight (mg.).						Adrenal weight (mg.).						Body weight (g.).					
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.	6.
Legs ties : 2½ months old. Died within 36 hrs.	297	201	335	322	219	228	44	24	24	34	32	32	107	114	124	116	113	106
Formaldehyde : 0·5 c.c. twice daily. Died within 36 hrs.	270	228	160	254	150	261	52	38	38	42	40	32	158	152	140	173	144	166
Adrenaline : 0·2 c.c. twice daily. Died after 24 hrs. 2½ months old	186	277	150	186	240	276	31	36	42	44	30	52	117	134	108	123	116	126
Hypophysectomized + cortin.																		
Adrenaline : 0·2 c.c. twice daily + cortin 0·2 c.c. 3 times per day, 48 hrs. 5 months old	120	110	176	98	125	135	41	33	31	32	40	34	159	180	160	150	129	115
Formaldehyde : 0·5 c.c. of 4 p.c. solution twice daily + cortin 0·2 c.c. 3 times per day, 48 hrs. 5 months old	137	99	110	138	81	89	50	40	41	42	38	40	165	167	176	145	170	160

after hypophysectomy, one would think that the cortex was essential for the involution of the thymus; but if it is recalled that surgical removal of the medulla will also inhibit thymus involution—even though a sufficient amount of cortical tissue is left intact—it would seem that both the cortex and the medulla have to be active in order to allow of thymus involution.

DISCUSSION.

The question arises: what is the probable significance of the reaction of the thymus and the adrenal to damaging influences?; for it cannot be mere coincidence that the adrenal and the thymus react first in such cases. The extensive studies made on the problem of excitement hyperglycæmia and the so-called "Fesselungs Hyperglykämie" of the German authors have undoubtedly showed already that adrenaline secretion occurs very easily even after mere psychic excitement. The fact that adrenaline secretion is stimulated by toxic doses of various drugs and that the processes of detoxification are interfered with in the absence of the adrenal have also long been known. If we compare our own findings with those described in the literature, we come to the conclusion that whenever the organism is placed in a critical situation it meets it with a typical defence reaction. This defence reaction consists of loss of body-weight, adrenal enlargement combined with loss of cortical lipoids, thymus œdema often combined with the formation of pleural transudate and sometimes retroperitoneal œdema. All these changes in the adrenal, the thymus, and water metabolism are to a certain degree under pituitary control because the pituitary stimuli are necessary to maintain the adrenal in a fully functional condition, while the adrenal, in turn, is essential in order to allow the thymus to respond. This defence reaction is most marked when a damaging influence acts on the organism for the first time. We saw repeatedly that, after an initial enlargement, the adrenal decreases in size and returns to normal even though the injections of the drug (such as morphine, atropine or adrenaline) which produced the initial enlargement are continued. In such cases the lipoid content of the cortex also returns to normal and the thymus resumes its normal size. It seems, therefore, that the changes in these glands are not due simply to the presence of the toxic substance in the organism, but are the morphological expression of an "alarm reaction" which enables the organism to meet critical situations efficiently. The significance of the changes in water metabolism, particularly of the tendency toward œdema formation, is not quite clear. The frequent occurrence of thymus œdema and the formation of pleural transudates show, however, that changes in water metabolism do occur during this alarm reaction of the organism, and we should like to mention in this connection that subcutaneous injection of formalin in the dose we used caused marked subcutaneous œdema in our adrenalectomized animals, while no such effect was seen in the other groups. The clinical conditions of drug habituation on the one hand, and the so-called toxic œdema and hunger œdema on the other, may have some bearing on this problem. Furthermore, it seems possible that unspecific fever therapy and protein therapy act by eliciting such an alarm reaction, which would help the organism to defend itself even against stimuli other than those which

elicited the reaction. We should like to point out that among all the damaging influences—toxic substances, surgical shock, exposure to cold, hunger, etc.—none failed to produce adrenal cortical hypertrophy if the experimental conditions damaged the animal seriously enough. This point is particularly important in view of the fact that many experimenters assay their pituitary preparations for adrenotropic activity on non-hypophysectomized animals. We must consider this method as unspecific, on the basis of the experiments mentioned in this paper, for any toxic extract will lead to cortical hyperplasia. Significant assays can only be made on hypophysectomized animals (Collip, Anderson and Thomson, 1933).

SUMMARY.

1. A syndrome consisting of rapid involution of the thymus, formation of a pleural transudate, and adrenal enlargement with loss of cortical lipoids, is produced in the rat by certain operative injuries, drugs, exposure to low temperature, and stimuli merely causing nervous excitement. In fact, among all the stimuli tried. none has been found which would seriously damage the experimental animal without causing rapid thymus involution and adrenal enlargement. Involution produced by such means is more pronounced than that which follows withdrawal of all food and water, and is therefore not due to failure of the animal to eat.

2. All the injuries and drugs which normally cause thymus involution are without effect on the size of the thymus of adrenalectomized animals. In hypophysectomized animals they have some effect, but much less than in normals.

3. A method for the isolated removal of the adrenal medulla with a suction pump is described. This method allows of removal of the medulla without seriously injuring the cortex. The thymus of rats thus deprived of medullary tissue but still in possession of active cortical tissue shows little if any ability to involute.

4. The inability of the thymus to undergo involution in adrenalectomized animals is not restored by sodium chloride, cortin in such doses as have been used, or adrenaline treatment.

5. Sodium chloride restores the otherwise low resistance of adrenalectomized rats to toxic doses of various drugs if the animals receive food. It is quite inactive, however, in the fasting animal.

6. The changes caused by a drug when it is given for the first time will subside later in spite of the continued administration of this drug. It appears, therefore, that the syndrome which we described consisting of thymus œdema followed by involution, formation of a pleural transudate, enlargement of the adrenal cortex with loss of its lipoid granules, and the accompanying rapid loss of body-weight, represent an " alarm reaction " which enables the organism to meet critical situations more efficiently. Attention is called to the possible connections between these experimental findings, and the clinical observations of drug habituation on the one hand and non-specific fever and protein therapy on the other hand.

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