rate of formation exists independently of the spatial disposition of the body. Bony fish, reptiles and amphibia accomplish this process by the action of lymph hearts. In higher mammals the system has evolved further: vulnerable lymph hearts as such are no longer present; instead all the lymphatic ducts are themselves pulsatile, and by virtue of the regular contractions of their muscular walls the lymph is propelled powerfully in a central direction. The evidence for the primacy of this mechanism has been published in detail[2], but perhaps it is worth quoting that in unanaesthetized sheep even small intermediate lymphatics generate regular pulse pressures of up to 10 mm of mercury. Thus the statement made by Dumont and Rifkind that lymph flow in mammals is dependent on "indirect and haphazard sources of energy" (for example, tissue tension and the movements transmitted from skeletal muscles and pulsating arteries) is untrue and arguments based on it are quite untenable. I am aware that the views of Dumont and Rifkind are in accord with those to be found in the standard texts[3,4], but these are now somewhat dated and like all texts contain a measure of self-perpetuating dogma for which there is no acceptable direct experimental evidence.

Of course, the point I have raised does not invalidate the principal conclusions of Dumont and Rifkind which I am sure will be well received by those interested in the lymphatic system and its evolution.

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Received September 23, 1968.

[1] Dumont, A. E., and Rifkind, K. M., *Nature*, **219**, 1182 (1968).
[2] Hall, J. G., Morris, B., and Woolley, G., *J. Physiol.*, **180**, 336 (1965).
[3] Yoffey, J. M., and Courtice, F. C., *Lymphatics, Lymph and Lymphoid Tissue* (Edward Arnold, London, 1956).
[4] Rusznyak, I., Földi, M., and Szabo, G., *Lymphatics and Lymph Circulation* (Pergamon Press, Oxford, 1960).

Selective Retention of Corticosterone by Limbic Structures in Rat Brain

CORTICOSTERONE is one of the principal steroids secreted by the rat adrenal gland[1–3] and it is well established that various noxious psychological and physiological stimuli promote the release of this substance into the blood. Because we are interested in the action of such corticosteroids on biochemical processes in the brain, we attempted to determine the degree to which circulating, radioactive corticosterone enters and remains in the brain. Furthermore, because the limbic system of the brain is implicated in the control of the secretion of ACTH[4–6] and in the affective behavioural responses[7–9], we were particularly interested to see whether structures in the limbic system retain corticosterone in a higher concentration than other areas of the brain. Published work on the uptake of the oestradiol by the brain[10–13] indicates that the hypothalamus retains that hormone.

Because of the possibility that endogenous corticosterone could interfere with the uptake of radioactive steroid by competing with it for sites in a limited-capacity retention mechanism, we have used adrenalectomized rats. Bilateral adrenalectomies were performed in our laboratory, between 1 and 3 weeks before the uptake experiment. Adrenalectomized animals were maintained on 0·8 per cent NaCl and standard lab chow given freely. Each animal was judged to be free of adrenal tissue, including accessory tissue[14], by assay of corticosterone in blood[15] obtained by heart puncture 30 min after the application of ether stress. Corticosterone-1,2-^{3}H (0·34 μg, 57·2 Ci/mmole; New England Nuclear Corp., Boston) was administered intraperitoneally in 50 μl. of 1 : 1 benzene ethanol and the rats were killed by decapitation 30 min, 2 h, 4 h or 6 h after the injection. A sample of blood (0·2 ml.) was obtained at decapitation. The brain was dissected according to an atlas of the rat brain[16], after consultation with a neuroanatomist, Dr George Wolf of Mount Sinai Hospital, New York, into the following regions: pituitary, medial plus lateral septum, hippocampus, hypothalamus, amygdala with overlying cortex, thalamus, brain stem (pons plus medulla oblongata) and cerebellum. The blood and tissue samples were extracted with dichloromethane (DCM) overnight on a metabolic shaker, which resulted in 92 per cent extraction of DCM-soluble radioactive material. Aliquots of this extract were counted with a liquid scintillation counter in a toluene-based scintillation cocktail. Details of the dissection, extraction and other aspects of the technical procedure will be presented elsewhere.

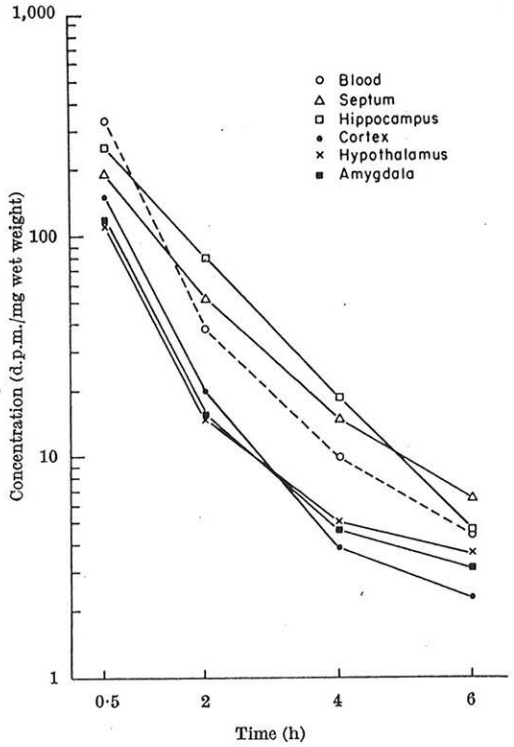

Fig. 1. Time course of disappearance of radioactivity administered as corticosterone-1,2-^{3}H from the blood and five regions of the brain of adrenalectomized rats. Each point is the average of determinations on four or five rats.

The entry of the radioactive hormone into the bloodstream and brain is very rapid, and concentrations of radioactivity have begun to decline by 30 min after administration. The disappearance of radioactivity from the blood and five regions of the rat brain is shown in Fig. 1, and is expressed as the concentration of radioactivity (DPM/mg wet weight) on a logarithmic scale against time up to 6 h after administration of the isotope. The concentration of radioactivity in cortex, hypothalamus and amygdala is about half of the blood concentration, and tends to decrease in parallel to the concentration in the blood, indicating a free and rapid exchange of labelled hormone between the blood and these regions of the brain. There is a slight tendency for the hypothalamus and amygdala to retain radioactivity at a level above that in the cortex beyond 4 h after administration of the isotope. The basic pattern, however, is similar to that of the cortex and is characteristic of virtually every region of the rat brain we have examined, with the striking exception of the septum and hippo-

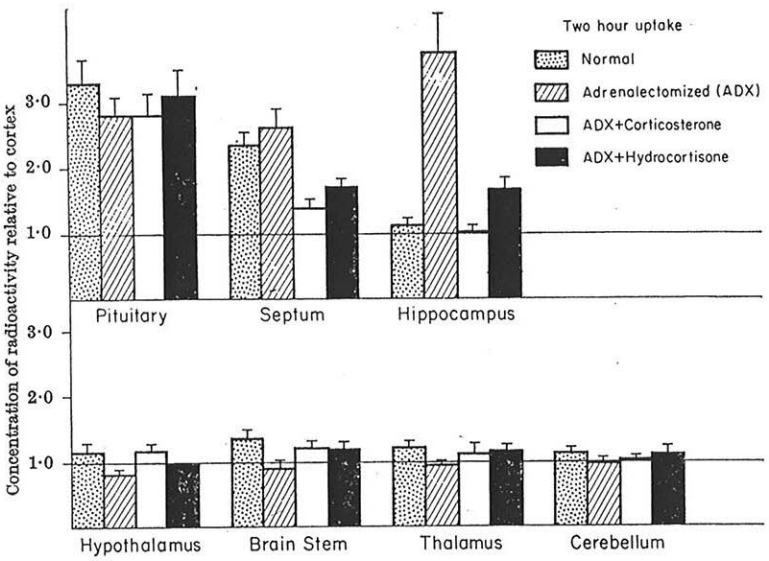

Fig. 2. Concentration of radioactivity in the pituitary and six regions of the rat brain divided by concentration of radioactivity in the cortex. Each bar represents the average (± *S.E.M.*) of determination on four or five rats. Uptake of radioactivity injected as corticosterone-1,2-^{3}H in normal rats is compared with that in adrenalectomized rats with and without prior administration of 3 mg of unlabelled corticosterone or hydrocortisone.

campus. These two regions take up and retain greater concentrations of radioactive material and release them at a different rate from the rest of the brain (Fig. 1).

We may be dealing with the occurrence in the septum and hippocampus of a hormone-binding mechanism analogous to the limited capacity binding sites which exist in the hypothalamus for oestradiol[10–13], and so we have begun to study the conditions in which the uptake and retention of radioactivity injected as corticosterone can be reduced or prevented. In particular, we have determined the extent to which exogenous corticosteroids and the endogenous steroids of normal rats are able to "saturate" the uptake process. A useful way of presenting data on the ability of unlabelled steroids to reduce the differential accumulation of corticosterone by the hippocampus and septum is to divide the concentration of radioactive material in these and other brain regions by that in the cortex. Fig. 2 shows that the hippocampal retention mechanism is "saturated" both by endogenous corticosterone in normal rats and in adrenalectomized rats by the administration of unlabelled corticosterone (3 mg in 0·2 ml. of ethanol given intraperitoneally 30 min before injection of labelled corticosterone). Hydrocortisone (3 mg in 0·2 ml. of ethanol, 30 min before the isotope) is less effective. The septal retention mechanism, by contrast, is not sensitive to the relatively low levels of endogenous corticosterone in normal rats, but is saturated by the high concentrations of exogenous corticosterone and to a lesser extent by hydrocortisone. The pituitary retains radioactive material, but this retention is not affected by endogenous or exogenous steroids. All other brain regions examined were unaffected by these treatments, and can be seen to have similar concentrations of radioactive material to the cortex.

Using thin-layer chromatography to separate corticosterone from other steroid metabolites[17], it has been possible to show that 2 h and 4 h after administration of radioactive corticosterone, 64 and 73 per cent respectively of the radioactive material extracted from the hippocampus is corticosterone. In the rest of the brain at the same time intervals, 60 and 56 per cent respectively is corticosterone. Although the brain has an enzyme which can convert corticosterone to 11-dehydrocorticosterone[18], we have found that less than 10 per cent of the radioactive material extracted from any region of the brain has the R_F value of 11-dehydrocorticosterone.

The functional role of the higher uptake and longer retention of corticosterone by the septum and hippocampus is so far unknown. It may be significant that, in the rat, the hippocampus[5,19] and the septum[4,20] have been shown to exert an inhibitory effect on the release of ACTH from the pituitary. It is impossible, however, to ascribe all actions of corticosterone on the brain to these two regions, for the hypothalamus, which does not retain the hormone, responds functionally and electrically to implantation of both synthetic and natural steroids[21–24]. It is tempting to suppose that the retention of hormone by the hippocampus and septum may be concerned with a particular type of steroid effect, such as the control of concentrations of certain enzymes in these two regions. This control might operate at the genetic level, for there is evidence for the accumulation and action of steroid hormones in cell nuclei of target tissues[25–29].

This work was supported by grants from the National Institutes of Health, US Public Health Service.

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Received September 7, 1968.

1 Bush, J. E., *J. Endocrinol.*, **9**, 95 (1953).
2 Davidson, C., *Endocrinology*, **54**, 580 (1954).
3 Reif, A. E., and Longwell, B. B., *Endocrinology*, **62**, 572 (1958).
4 Bohus, B., *Acta Physiol. Hung.*, **20**, 373 (1961).
5 Knigge, K. M., *Proc. Soc. Exp. Biol. and Med.*, **114**, 67 (1963).
6 Usher, D. R., Kasper, P., and Birmingham, M. K., *Neuroendocrinology*, 2, 157 (1967).
7 Wheatley, M. D., *Arch. Neurol. Psychiat.*, **52**, 296 (1944).
8 Kaada, B., *Acta Physiol. Scand.*, **24**, Suppl. 83 (1951).
9 Brady, J. V., and Nauta, W. J., *J. Comp. Physiol. Psychol.*, **46**, 339 (1953).
10 Eisenfeld, A. J., and Axelrod, J., *J. Pharmacol. Exp. Therap.*, **150**, 469 (1965).
11 Eisenfeld, A. J., and Axelrod, J., *Endocrinology*, **79**, 38 (1966).
12 Eisenfeld, A. J., *Biochim. Biophys. Acta*, **136**, 498 (1967).
13 Kato, J., and Villee, C. A., *Endocrinology*, **80**, 567 (1967).
14 Richter, C. P., *Endocrinology*, **29**, 115 (1941).
15 Peterson, R. E., *J. Biol. Chem.*, **225**, 25 (1957).
16 König, J. F. R., and Klippel, R. A., *The Rat Brain* (Williams and Wilkins, Baltimore, 1963).
17 Lisboa, B. P., *Acta Endocrinol.*, **43**, 47 (1963).
18 Grosser, B. I., *J. Neurochem.*, **13**, 475 (1966).
19 Knigge, K. M., *Abstracts, Second Intern. Cong. Horm. Steroids, Milan*, 208 (1966).
20 Dallman, M. F., and Yates, F. E., *Mem. Soc. Endocrinol.*, **17**, 39 (1968).
21 Endroczi, E., Lissak, K., and Tekeres, M., *Acta Physiol. Hung.*, **18**, 291 (1961).
22 Davidson, J. M., and Feldman, S., *Endocrinology*, **72**, 936 (1963).
23 Smelik, P. G., *Acta Physiol. Pharm. Neerland*, **13**, 371 (1965).
24 Ruf, K., and Steiner, F. A., *Science*, **156**, 667 (1967).
25 Jensen, E. V., and Jacobson, H. I., *Rec. Prog. Hormone Res.*, **18**, 387 (1962).
26 Notebloom, W. D., and Gorski, J., *Arch. Biochem. Biophys.*, **111**, 559 (1965).
27 Barton, R. W., and Liao, S., *Endocrinology*, **81**, 409 (1967).
28 Bruchevsky, N., and Wilson, J. D., *J. Biol. Chem.*, **243**, 2012 (1968).
29 Teng, C.-S., and Hamilton, T. H., *Proc. US Nat. Acad. Sci.*, **60**, 1410 (1968).

Changes of Length within the Frog Muscle Spindle during Stretch as shown by Stroboscopic Photomicroscopy

The impulse response of the muscle spindle to an applied stretch consists of a dynamic discharge during the phase of increase in length, followed by a static discharge while stretch is maintained at a steady level[1–3]. The underlying receptor potential shows corresponding characteristics : a rapid dynamic rise followed by a decay to a static level (Fig. 1)[2,4]. This differentiation of the response