

branch, as suggested by Allison, which play their part in the extremely short life-spans of chick embryo erythrocytes. One of these factors might be the high degree of immaturity at which the chick embryo erythrocytes are poured out into the blood stream<sup>2</sup>.

A full account of our calculations is to be published elsewhere<sup>3</sup>.

Future investigations will show if this relatively small number of recirculations holds good also for mammalian primitive erythrocytes. There are indications that their average life-spans are also about one-quarter of that found in adult mammals<sup>10</sup>.

L. LEMEŽ  
Z. RYCHTER

Department of Anatomy,  
Charles University, Prague.

<sup>1</sup> Allison, A. C., *Nature*, **188**, 37 (1960).

<sup>2</sup> Lemež, L., and Kopecký, M., *Physiologia Bohemoslovenica* (in the press).

<sup>3</sup> Hughes, A. F. W., *J. Roy. Micro. Soc.*, **69**, 145 (1949).

<sup>4</sup> Romanoff, A. L., *The Avian Embryo. Structural and Functional Development* (The Macmillan Co., New York, 1960).

<sup>5</sup> Rychter, Z., Kopecký, M., and Lemež, L., *Československá morfologie*, **3**, 11 (1955); *Nature*, **175**, 1126 (1955).

<sup>6</sup> Lemež, L., and Rychter, Z., *Československá morfologie*, **4**, 27 (1956).

<sup>7</sup> Lemež, L., *Československá morfologie*, **6**, 323 (1958). Lemež, L., and Rychter, Z., *Anat. Anzeiger* (Ergh.), **105**, 315 (1959).

<sup>8</sup> Ottesen, J., *The Life Cycle of Hen Erythrocytes* (Munksgaard, Copenhagen, 1955).

<sup>9</sup> Lemež, L., *Československá morfologie*, **1**, 35 (1953).

<sup>10</sup> Lemež, L., *Československá morfologie*, **5**, 201 (1957).

## PHARMACOLOGY

### Stimulation of Uptake of Calcium-45 in the Adrenal Gland by Acetylcholine

RECENTLY, Douglas and Rubin<sup>1</sup> have suggested that acetylcholine, the physiological transmitter of sympathetic nerve effects at the adrenal medulla, evokes catecholamine secretion by causing calcium ions to penetrate the medullary cells. Our experiments with calcium-45 have now provided further evidence consonant with this idea.

Adrenal glands from nine cats were perfused *in vitro* with Locke's solution. One gland from each animal was used to determine 'resting' uptake of calcium and the contralateral gland stimulated with acetylcholine. Both glands were perfused with Locke's solution containing calcium-45 for about 10 min. during which time one was given acetylcholine in a concentration ( $10^{-5}$  gm./ml.) sufficient to cause a brisk secretion of catecholamines. Thereafter, they were perfused with Locke's solution containing only calcium-40 and measurements were made of the efflux of calcium-45. Loss of calcium-45 from the perfused glands was at first very rapid, but it slowed after about 50 min. and the decline in activity in the glands thenceforth followed a simple exponential course. It was assumed, on the basis of the work of others on muscle<sup>2,3</sup>, that by this time the extracellular calcium-45 had been completely washed out. One hundred minutes after beginning the washout, stimulated and unstimulated glands were separated into medulla and cortex and their radioactivity determined.

The glands exposed to acetylcholine contained more radioactivity than the unstimulated glands. On a weight basis, the medulla was several times more active than the cortex. Comparison of the stimulated and 'resting' medulla indicated that during perfusion with acetylcholine the rate of calcium uptake was increased about eight-fold. A number of similar experiments were carried out with potassium, which is also

known to evoke adrenal secretion. Potassium (56 mM) also caused an increased uptake of calcium-45, with the medulla again showing the greatest rate of uptake. This stimulant effect of potassium on calcium influx persisted in the presence of hexamethonium sufficient to suppress indirect effects due to stimulation of nerve endings present in the gland, and was presumably due to depolarization of the medullary cells.

We observed incidentally that acetylcholine and potassium also increased the rate of uptake of calcium-45 by the cortex, although to a lesser extent. This effect was too great to be accounted for by incomplete separation of medulla from cortex. It may be related to a stimulant effect of acetylcholine on adrenocortical secretion, such as Rosenfeld<sup>4</sup> observed in perfused calf adrenals.

The finding of increased uptake of calcium during exposure to acetylcholine or potassium is consistent with the idea that acetylcholine evokes catecholamine secretion by causing calcium ions to penetrate the adrenal medullary cells and that this penetration may be brought about by depolarization<sup>1</sup>. The finding also extends the remarkable parallelism previously noted<sup>1</sup> between the two sets of evidence implicating calcium in 'stimulus-secretion coupling' at the adrenal gland and in 'excitation-contraction coupling' in muscle.

W. W. DOUGLAS  
A. M. POISNER

Department of Pharmacology,  
Albert Einstein College of Medicine,  
Eastchester Road and Morris Park Avenue,  
New York, 61.

<sup>1</sup> Douglas, W. W., and Rubin, R. P., *J. Physiol.*, **159**, 24 P (1961); *Biochem. Pharmacol.*, **8**, 20 (1961). Douglas, W. W., and Rubin, R. P., *J. Physiol.*, **159**, 40 (1961).

<sup>2</sup> Harris, E. J., *Transport and Accumulation in Biological Systems*, second ed. (Butterworths, London; Academic Press, New York, 1960).

<sup>3</sup> Shanes, A. M., *J. Cell. Comp. Physiol.*, **57**, 193 (1960).

<sup>4</sup> Rosenfeld, G., *Amer. J. Physiol.*, **183**, 272 (1955).

### Pharmacodynamical Effects of Asarone and $\beta$ -Asarone

DANDIYA *et al.*<sup>1,2</sup> have determined the effect on the central nervous system of *Acorus calamus*, a drug used in the Indian system of medicine for various ailments including some of the mental disorders. In the present work two fractions 'asarone' and ' $\beta$ -asarone' (isolated by Baxter and Kandel) of the volatile oil of *Acorus calamus* have been investigated with regard to effects on the central nervous system and other pharmacodynamical actions which these drugs have.

The prolongation of hypnotic activity of (i) pentobarbitone, (ii) hexobarbitone, and (iii) ethanol was investigated in white mice. Asarone and  $\beta$ -asarone were given in different groups of animals intraperitoneally in doses of 50 mgm./kgm., followed 15 min. later by one of the anaesthetic agents mentioned. The sleeping time was recorded in the manner described in refs. 1 and 2. It was observed that both asarone and  $\beta$ -asarone significantly ( $P < 0.01$ ) prolonged the sleeping time of all the anaesthetic agents mentioned, for example, the mean sleeping time due to pentobarbitone alone, of  $101.6 \pm 14.5$  min., was raised to  $254.5 \pm 18.8$  min. when the animals were pretreated with  $\beta$ -asarone and to  $206.4 \pm 28.3$  min. when the animals were pretreated with asarone.

It was also observed that the hypnotic potentiating property of  $\beta$ -asarone was significantly more ( $P < 0.05$ ) than that of asarone in these doses against all these anaesthetic agents. Asarone or