imino group of proline is one terminal group of salmin and the carboxyl of arginine the other. Tristram[2] mentions unpublished results of Porter using the dinitrofluorobenzene method as strong evidence that proline is the end-group of salmin. This may account for the absence of the ninhydrin color of these protamines.

The data obtained in this study indicate that the ratio of arginine to monoamino acids in both salmin and clupein is somewhat higher (*cf.* [1]) than the 2:1 ratio postulated by Kossel.[3]

The question of homogeniety of salmin was discussed in a previous publication.[1] A similar situation holds with respect to clupein. Since that time, numerous unsuccessful attempts to fractionate salmin and clupein have been made from 90% aqueous isopropanol at 90°C, 25°C, and 0°C, and by paper chromatography using water-saturated phenol, lutidine-water-alcohol, water alone, 0.1 *N* H_2SO_4 and other solvents. The dinitrophenyl derivatives of the protamines also failed to yield more than one spot on paper chromatograms using various solvents. Although these preparations may be chemically inhomogeneous, their overall physical properties must be rather similar due to the preponderance of arginine.

Summary. A simplified procedure for the preparation of salmin and clupein is described. The amino acid content of clupein as well as salmin has been estimated.

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Effect of Electronarcosis on Level of "Adrenalin-like" Compounds in Blood.

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A chemical method for the quantitative determination of adrenalin was devised by Whitehorn.[1] This procedure was modified by Shaw[2,3] who showed that the method is not specific for this compound, and that a number of substances related to adrenalin give the same reaction. Shaw's method was applied to blood by Tietz, Dornheggen and Goldman,[4] Raab,[5] Bloor,[6] and others. It became apparent that the material thus estimated is not adrenalin alone, but that other compounds determined by Shaw's procedure are present in the blood.

Previous observations showed that the level of these "adrenaline-like" compounds is increased in the blood of patients during insulin shock.[7] It was also found to be increased during electronarcosis and electroshock treatment in humans.[8] In the present paper the effect of electronarcosis on the level of these compounds was investigated experimentally.

Methods. The technic used for the estimation of adrenalin-like compounds in blood has been reported before.[7] In order to limit the total amount of blood taken from the animal the method was adapted in the following way to the use of about 2 cc per determination. Blood was taken from the jugular vein which had been exposed previously under local anesthesia. A little more than 2 cc of blood was drawn from the vein in an iced syringe and squirted immediately into a small tube cooled in ice. Of this, 2 cc was measured

1 Whitehorn, C., *J. Biol. Chem.*, 1935, **108**, 633.

2 Shaw, F. H., *Biochem. J.*, 1938, **32**, 19.

3 Shaw, F. H., *Australian J. Exp. Biol. and Med. Sc.*, 1946, **24**, 53.

4 Tietz, E. B., Dornheggen, H., and Goldman, D., *Endocrinology*, 1940, **26**, 641.

5 Raab, W., *Endocrinology*, 1941, **28**, 325.

6 Bloor, W. R., *J. Biol. Chem.*, 1939, **128**, ix.

7 Tietz, E. B., and Birnbaum, S. M., *Am. J. Psych.*, 1942, **99**, 75.

8 Tietz, E. B., unpublished data.

with an iced pipette in a test tube and mixed with 4 cc 10% trichloracetic acid. After 30 minutes 8 cc 5% trichloracetic acid was added and after inverting the tube twice the contents were filtered. The filtrate was kept in the ice box and 2 cc were used for each determination. The amount of adrenalin-like compounds was expressed as the adrenalin chloride equivalent (in gammas/100 cc of blood).

Electronarcosis was produced by applying a 60-cycle alternating current to the brain through electrodes placed on both sides of the head directly behind the eyes. Electronarcosis was started with a relatively high current 150-200 mA, which was continued for 30 seconds. The current was then dropped to 25-40 mA after which respiration was reestablished. This current was increased or decreased as needed to maintain a quiet electronarcosis.[9] The total duration of electronarcosis in the present experiments was limited to 5 minutes. Rabbits were used exclusively.

Results. In order to establish a control two blood samples were taken with a 10-minute interval before electronarcosis. In 33 experiments the mean of the level of adrenalin-like compounds in the first of these samples was 21 gamma/100 cc; the individual values varying between 17 and 25 gamma/100 cc. The difference between the two control values supplies information about the variability of the determination and about changes in the concentration of the adrenalin-like compounds during a 10-minute period. The mean difference in these 33 experiments was 0.03 gamma/cc, the largest difference was 3 gamma/cc. Using a different modification of the Whitehorn procedure, Kobro[10] obtained comparable values for the adrenalin-like compounds in rabbit's blood.

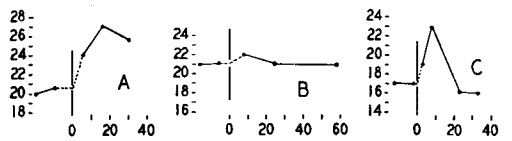

FIG. 1.

The level of adrenalin-like compounds (in gammas per 100 cc) is plotted against time in minutes. The vertical line in A indicates the beginning of a 5-minute electronarcosis, in B the same in a curarized and vagotomized preparation, and in C the beginning of a 2½-minute asphyxiation in a curarized preparation.

In agreement with the observations on patients the level of adrenalin-like compounds rose during electronarcosis (Fig. 1, A). In 9 experiments the mean of the maximal rise in the level was 5 gamma/100 cc. The mean difference between the two control determinations in these 9 experiments was 0.2 gamma/100 cc. Statistical treatment of these data shows that the increase of the level due to electronarcosis is significant at the 5% level ($t = 2.7$).

As shown in Fig. 1, A, the level of adrenalin-like compounds has started to decline again 30 minutes after the end of electronarcosis. It is of interest to note that Kobro[10] observed a fall of the level 30 minutes after an injection of adrenalin.

The initial stage of electronarcosis is characterized by a moderately severe asphyxiation of the animal and by convulsions. Both concomitants can be prevented by the administration of Intocostrin (0.5-0.8 cc/kg) combined with artificial respiration. Furthermore such preparations were doubly vagotomized to prevent the cardiac arrest which usually occurs in the beginning of electronarcosis[9] and which might cause a short systemic asphyxiation. The effect of electronarcosis on the level of adrenalin-like compounds in such preparations was variable, but in general an unusually small rise of the level was found. The results of one of these experiments are given in Fig. 1, B.

Since there was some indication that either convulsive activity or asphyxiation (or both) might cause the increase of adrenlin-like compounds during electronarcosis, the effect of asphyxiation alone was examined. In curarized preparations the trachea was clamped for a total of 2½ minutes (usually with a short interval when the heart action became weak). This caused a rise in the level of the compounds which was of the same order as the increase observed after

[9] Frostig, J. P., van Harreveld, A., Reznick, S., Tyler, D. B., and Wiersma, C. A. G., *Arch. Neurol.*, 1944, **51**, 232.

[10] Kobro, M., *Acta Med. Scand.*, 1946, **125**, 49.

electronarcosis. Fig. 1, C, shows the results of such an experiment. The mean increase of 4 experiments was 6 gamma/100 cc. In the blood of animals killed by asphyxiation the level rises even more, up to 38 gamma/100 cc has been observed.

Discussion and Summary. There is reason to believe that when asphyxiation is prevented electronarcosis causes only small increases of the level of adrenalin-like compounds in blood. It had been found previously[11] when one of two rabbits connected by a double carotid-jugular anastomosis was given an asphyxia-free electronarcosis, that the blood pressure in the other showed a slight increase. This was believed to be due to a release of adrenalin, which in the present experiments may cause the slight rise in the level of "adrenalin-like" compounds during asphyxia-free electronarcosis. It seems likely that the much larger increase of these compounds which is always present in animals subjected to electronarcosis without further precautions, is caused by asphyxiation.

Kobro[12] did not observe any effect of respiratory impairment (letting the animal breathe through a tightly woven cloth) on the adrenalin level. The impairment employed in the present experiments, though of shorter duration, was more severe (clamping of the trachea), which may account for the difference in the results obtained.

[11] van Harreveld, A., and Dandliker, W. B., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 391.

[12] Kobro, M., *Acta Med. Scand.*, 1946-47, **126**, 97.

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Obesity in Albino Mice Due to Single Injections of Goldthioglucose.

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During a toxicological study of goldthioglucose, it was noted that a number of albino mice receiving this compound showed a marked increase in size. This response occurred in animals which had been injected with a single dose of this drug. The present investigation was undertaken in an attempt to define the conditions under which these gains in weight occur.

Experimental. Goldthioglucose is an unstable compound of gold and thioglucose, containing 50% gold. It dissociates in the presence of water. The compound is hygroscopic and is therefore utilized as a suspension in sesame oil. The concentration of the suspension used in these experiments was 100 mg of goldthioglucose in 1 cc of the oil (supplied by Schering Corporation, Bloomfield, N.J.). The route of administration was by intraperitoneal or intramuscular injection.

Stock male and female mice were used. The animals were kept on a pellet diet, and fed *ad lib.* In some of these studies the animals were kept in individual cages and the food intake determined. All animals were weighed at weekly intervals.

The LD_{50} of goldthioglucose was 40-50 mg intraperitoneally for mice weighing 20 g. Fifty to 70% of the animals survived doses of 35 mg of goldthioglucose, and 90% survived doses of 25 mg. All animals survived the intraperitoneal injection of 12 mg of the compound. Fatalities from the administration of goldthioglucose generally occurred within the first 3 to 4 days. There was no further mortality due to the toxicity of the drug among those animals which survived one week.

In one representative experiment, animals weighing 18-23 g were given single injections