FIG. 2. Photograph of chromatogram from hydrolyzate of mouse pancreas mitochondria.

run on each sample, and comparisons between samples were made for chromatograms on which the amino acids occurring in the highest concentrations gave spots of approximately equal area and intensity, as shown in Figs. 1 and 2. Mitochondrial fractions of the liver and kidney of the mouse and rat, and of pancreas, mammary gland, hepatoma, squamous cell, and mammary carcinomas in the mouse were analyzed.

Photographs of two typical chromatograms are shown in Figs. 1 and 2. The visible constituents are identified on Fig. 1. The yellow spots given by proline and hydroxyproline are not visible on the photographs because of insufficient contrast, although they were visible on the original chromatograms. Eighteen amino acids were clearly identified on the chromatograms. Tryptophan was not observed because of destruction during acid hydrolysis. Glutamic acid, aspartic acid, glycine, alanine, serine, proline, the leucines, and valine were present in the largest quantities. Somewhat smaller amounts of lysine, arginine, threonine, phenylalanine, and tyrosine were found. Methionine, cystine, histidine, and hydroxyproline were present in the smallest concentrations.

There was a remarkable constancy in the relative amounts of the chief amino acids found in the mitochondria from the different samples of tissue examined. This is illustrated in Figs. 1 and 2, in which preparations from different organs of different species are compared. The chromatograms suggest the possibility that the rat kidney mitochondria might contain slightly more lysine and histidine and slightly less methionine than those from mouse pancreas. This will have to be checked by quantitative procedures. The patterns of amino acids found in the preparations from the malignant tissues examined were virtually identical with those obtained from normal mouse tissues.

The similarity of the amino acid patterns found in the mitochondrial fractions of the various tissues studied suggests that there is a characteristic protein, or combination of proteins, associated with these particles. The results also indicate that the quantity of the dicarboxylic amino acids exceeds that of the basic amino acids.

References

1. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*; 1944, **38**, 224.
2. DENT, C. E. *Biochem. J.*, 1948, **43**, 169.
3. HOGEBOOM, G. H., SCHNEIDER, W. C., and PALLADE, G. E. *J. biol. Chem.*, 1948, **172**, 619.

On the Participation of the Reticulo-endothelial System in the Alarm Reaction

P. S. Timiras and H. Selye

Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montréal, Canada

We wish to report upon experiments which revealed a marked increas in phagocytic activity of the reticulo-endothelial system during the alarm reaction, that is, during the first phase of the general adaptation syndrome.

The reticulo-endothelial system probably participates in the defense of the organism through its phagocytic activity, and by production of antibodies, agglutinins, antitoxins, etc. (*1, 4*). A local increase in the activity of this system has been described in the thymus (*5*) after exposure to various acute nonspecific stresses, and in the spleens of rats subjected to chronic inanition (*3*) or injected with cortical extracts (*2*). Stimulated by these findings, we have performed two series of experiments in order to clarify the relationship between the reticulo-endothelial system and the hormonal and metabolic changes which occur during the alarm reaction.

In our first experiment, 44 piebald male rats (average body weight 150 g) were divided into four equal groups: group I served as untreated controls; groups II, III, and IV were fasted 48 hr, and during the last 24 hr were submitted to various stresses, such as cold (0 – 5° C), spinal cord transection (at the height of the 7th cervical vertebra), and repeated, exhaustive, forced exercise. All animals were injected intravenously with 2 ml of a dilute solution of Higgins India ink (1 part India ink to 5 parts physiologic NaCl solution) 1 hr before they were killed. At autopsy, naked eye inspection showed, in all the stressed animals, a markedly increased deposition of India ink in the lung, kidneys, adrenals, bone marrow, and the "hibernating gland." In the hibernating gland, this was accompanied by an acute discharge of lipid granules, hyperemia, and edema. These changes are characteristic of the alarm reaction and have been given special attention elsewhere (*6*). Compared to the controls, the India ink deposition in the liver of the alarmed rats did not seem to be significantly increased, while in the spleen there was a diminution of India ink deposition.

Subsequent histological examination confirmed and extended the autopsy findings. There was increased phagocytosis in the lung, kidneys, adrenals, bone marrow, hiber-

nating gland, thymus, and lymph nodes. Phagocytosis was not increased in the hepatic macrophages of the alarmed animals. In the spleen, which undergoes marked atrophy during the alarm reaction, there was a slight decrease in the India ink phagocytosis.

In a second series of experiments, 20 piebald male rats (average body weight 130 g) were divided into four groups and treated as in the first experiment. The autopsy and histologic findings confirmed the observations already described.

The results of our experiments suggest an active participation of the reticulo-endothelial system in the defense of the organism during the alarm reaction.

References

1. CHEVREMONT, M. *Biol. Rev.*, 1948, **23**, 267.
2. GORDON, A. S. *Fed. Proc.*, 1946, **5**, no. 1.
3. GORDON, A. S. and KATSH, G. F. *Fed. Proc.*, 1949, **8**, no. 1.
4. PERLA, D. and MARMORSTON, J. *The spleen and resistance.* Baltimore: Williams and Wilkins, 1935.
5. SELYE, H. *J. clin. Endocrinol.*, 1946, **6**, 117.
6. SELYE, H. and TIMIRAS, P. S. *Nature*, Lond., 1949, **164**, 745.

Colorimetric Estimation of Noradrenalin in the Presence of Adrenalin

U. S. von Euler and Ulla Hamberg

Karolinska Institutet, Stockholm, Sweden

Since noradrenalin generally occurs in animal tissues in a variable mixture with adrenalin (*1, 3, 5*), it has become desirable to find methods for quantitative assay of both substances in a mixture. This can be done by taking advantage of the difference in activity ratio of noradrenalin and adrenalin on various test objects, such as the blood pressure of the cat in chloralose anesthesia, and the hen's rectal cecum (*3, 5*). Although the general type of effect is the same, noradrenalin may have 2–4 times the effect of adrenalin on the cat's blood pressure weight for weight, but only 1/10–1/50 of the activity of adrenalin on the fowl's rectal cecum. By computing the results of the assay, the relative amounts of noradrenalin and adrenalin may be estimated with a fairly high degree of accuracy. Though the biological method has the advantage of high specificity and requires only small amounts of active material, it is time-consuming and necessitates the use of at least two test animals. For this reason a simpler chemical method has been developed. The procedure given herein has been adopted as satisfactory.

The biological material is purified either by adsorption on alumina, according to the method of Shaw, modified by von Euler (*4*), or by the method of Bergström and Hansson (*2*), based on the ion-exchange principle.

The colorimetric method is based on the formation of noradrenochrome and adrenochrome on oxidation with iodine. The adrenochrome formation is complete when iodine is allowed to act for 1½ min at pH 4.0, whereas only about 10% of the noradrenalin is transformed into noradrenochrome under the same conditions. On 3-min treatment with iodine at pH 6.0, maximal formation of noradrenochrome and adrenochrome is attained.

The procedure is as follows: To an amount of purified extract containing 20–200 μg catechol derivatives, 1 ml *n*-acetate buffer of pH 4 and 0.2 ml of 0.1 *N* iodine solution is added. After precisely 1½ min, excess iodine is removed with 0.05 *N* sodium thiosulfate. The color is read within 5 min against a blank without iodine in a photometer at wavelength 529 mμ. The procedure is repeated with a second sample using acetate buffer pH 6 and 3-min iodine treatment. Standard readings are made with 100 μg adrenalin and noradrenalin at pH 4 (1½ min), and pH 6 (3 min), giving the calibration factors for both substances and the percentage of noradrenalin oxidized at pH 4 in 1½ min. At 529 mμ, the adrenochrome figure is the same on oxidation at pH 4 and pH 6.

Computation of results:

a = reading at pH 4 (1½-min iodine treatment)

b = " " pH 6 (3- " " ")

m = calibration factor for adrenalin $\left(\frac{100}{\text{reading for } 100\ \mu g}\right)$

n = " " " noradrenalin "

p = relative amount of noradrenalin oxidized at pH 4 in 1½ min

$$\begin{cases} \text{noradrenalin} = n \cdot \dfrac{b-a}{1-p} \\ \text{adrenalin} = m \left[a - p \dfrac{(b-a)}{1-p} \right] \end{cases}$$

With mixtures of noradrenalin and adrenalin in varying proportions, the results indicated in Table 1 were obtained.

TABLE 1

Amounts added:		Calculated from readings:	
adrenalin	noradrenalin	adrenalin	noradrenalin
μg	μg	μg	μg
10	90	11.8	86.8
20	80	20.5	80.5
30	70	30.3	68.6
40	60	40.8	59.5
50	50	50.7	47.7
60	40	61.1	39.5
70	30	70.0	30.4
80	20	79.7	19.5
90	10	90.8	9.6

The method has been repeatedly tested on purified suprarenal extracts and results have agreed well with those obtained from biological methods.

References

1. BACQ, Z. M. and FISCHER, P. *Arch. intern. Physiol.*, 1947, **55**, 73.
2. BERGSTRÖM, S. and HANSSON, G. *Acta Physiol. Scand.* In press.
3. VON EULER, U. S. *Nature*, 1948, **162**, 570.
4. ———. *Arch. int. Pharmacodyn.*, 1948, **77**, 477.
5. GOODALL, MCCH. *Acta Physiol. Scand.* In press.