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.

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Colorimetric Estimation of Noradrenalin in the Presence of Adrenalin

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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.

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