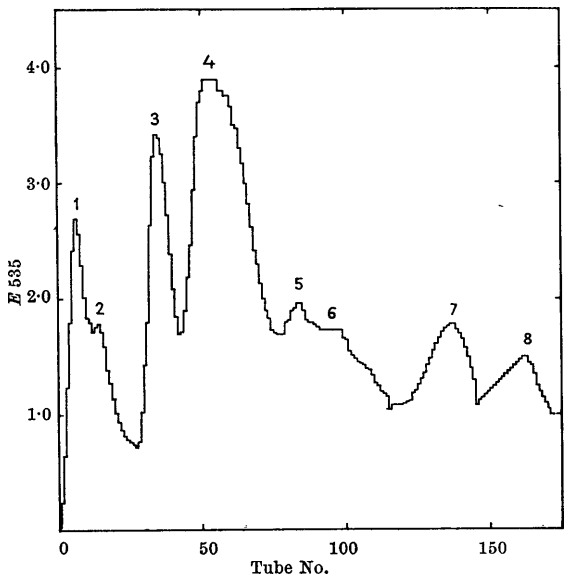

Fig. 1. Fractionation of the anthocyanins of the blueberry (*Vaccinium myrtillus* L.) using a cellulose column filled with Whatman cellulose powder and *n*-butanol/2 *N* hydrochloric acid (1 : 1) as developing solvent

in a column (5 cm. × 110 cm.) filled with cellulose powder (Whatman cellulose powder, standard grade or 'Linterspulver' No. 124, Schleicher and Schüll), *n*-butanol/2 *N* hydrochloric acid (1 : 1) being used as the solvent system[4]. The eluate was collected in 10-ml. fractions and the colour was measured at 535 mμ. From a graph with plots of extinction values (Fig. 1), it was found that the anthocyanins of the blueberry separate out into 8 peaks.

Samples of the eluates of peaks 3 and 4, which correspond to the anthocyanins of reddish blueberries, were hydrolysed by heating. The R_F values obtained by paper chromatography of the liberated anthocyanidins are shown in Table 1. The results establish that the aglycones of both anthocyanin fractions can be identified as cyanidin. This was confirmed by the distribution and oxidation tests and tests with the cyanidin reagent, sodium acetate and ferric chloride (Robinson's method).

The sugars were determined from the hydrolysed fractions 3 and 4. The water extracts were concentrated to about 10 ml., the hydrochloric acid was eliminated by extracting with 10 per cent di-*n*-octylmethylamine containing chloroform, and the solution was further concentrated to 1 ml. The sugars were identified by paper chromatography (*n*-butanol/acetic acid/water, 4 : 1 : 5), and it was observed that fraction 3 contained glucose and arabinose, fraction 4 glucose only. Control experiments proved that the arabinose was not an artefact produced either from the Whatman cellulose powder or from the paper (see ref. 5). Aniline–citric acid reagent was used in the quantitative determinations of the sugars. Comparison of the separately determined concentrations of the aglycones with the sugar concentrations proved that the concentration of glucose in both fractions was in almost equimolar proportion to the concentration of the anthocyanidin. The amount of arabinose, however, was found to be only half the equivalent amount, possibly owing to disintegration or partial splitting.

Table 2 shows the values obtained by paper chromatographic analysis of the anthocyanins. The R_F values of the anthocyanin in fraction 4, the largest fraction, in different solvent systems was found to correspond to the R_F values of cyanidin-3-glucoside. Considering the equivalent proportions of the aglycone and the sugar, the anthocyanin must be cyanidin-3-glucoside. In fraction 3 the R_F values of the arabinose-containing cyanidin derivative do not—as expected—agree with the R_F values of the reference preparations. According to the literature, however, the R_F values of this fraction closely approach the migration of cyanidin-3-glucopentosides.

In conclusion, the two anthocyanins initially appearing during the maturation process of blueberries are both found to be cyanidin derivatives. These two cyanidin derivatives, the first to appear being cyanidin-3-glucoside, and the arabinose-containing cyanidin derivative (possibly cyanidin arabinoglucoside), are the most abundant pigments of ripe blueberries, although it is known that the ripe berries also contain glycosides of malvidin, delphinidin and petunidin[1]. The order of appearance of these pigments during the ripening process bears out the earlier concept of Robinson[6] that biogenetically cyanidin should be regarded as the simplest of the anthocyanidins.

We thank Dr. C. G. Nordström for the preparation of cyanidin-3,5-diglucoside which he kindly placed at our disposal.

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PHYSIOLOGY

Effect of Administration of Lysergic Acid Diethylamide on Serotonin-Levels in the Body

In a previous publication[1], we have suggested that some of the effects of lysergic acid diethylamide (LSD) may be mediated through its effect on the serotonin content of brain. Brodie and his associates[2] have previously reported that LSD—and also some other drugs—do not release serotonin as reserpine does. However, Freedman[3] has recently reported slightly raised levels of serotonin in the whole brain of rats treated with LSD. Using animals which had been given reserpine, they found a twofold increase of brain serotonin in rats treated with LSD over the controls receiving reserpine alone. The whole brain serotonin may not be a true indication of the action of a psychopharmacological agent. Thus Vogt and her associates[4] have reported different effects of LSD and serotonin in different areas of the brain after intraventricular injection.

We have investigated the effect of LSD on the serotonin content of the different parts of the rabbit brain. The brain was divided into four major parts : (1) cerebral hemispheres, excluding midbrain, pons, thalamus, hypothalamus, etc., and mostly consisting

of the grey and white matter; (2) cerebellum; (3) stem; and (4) rest of the brain.

The animals were given intravenously 500 μgm. of LSD and were killed 15 min. later. In a second series of investigations, the rabbits were given intravenously 0·92 mgm., equal to 10 μc., of 5-hydroxytryptophan-3-^{14}C (5-HTP) followed by 500 μgm. of LSD 45 min. later. The experimental animals were killed 15 min. after the administration of LSD, while the controls received no LSD and were killed 1 hr. after the administration of the 5-HTP. Since the nature of the results as to the effect of LSD on the serotonin content was similar in both series of investigations, the results of the second series only are reported here. Table 1 presents the results as an average of four to six animals for each group. The first column represents the amount of total radioactivity per gm. of tissue and consists of the radioactivity due to 5-HTP, serotonin and their metabolites. The second column presents the content of serotonin determined spectrophotofluorimetrically, while the third one shows the specific radioactivity of the serotonin. The experimental details will be presented in detail elsewhere.

Table 1. Effect of Administration of Lysergic Acid Diethylamide on the Levels of Serotonin in the Body

Tissue	Total radioactivity (counts per min. per gm. tissue)		Serotonin (μgm. per gm. tissue)		Specific radioactivity of serotonin (counts/min./μgm.)	
	Control	LSD	Control	LSD	Control	LSD
Liver	884	665	1·74	2·62	14·3	15·0
Lung	545	439	1·02	1·46	7·0	3·0
Kidney	1,992	3,089	1·04	1·78	17·0	25·0
Spleen	1,753	1,713	42·14	55·14	1·8	2·3
Heart	277	346	1·34	3·86	—	3·3
Cerebrum	76	83	0·96	0·90	1·2	5·7
Cerebellum	112	450	1·24	1·72	0·7	2·1
Stem	108	273	2·12	2·94	—	6·8
Rest of brain	132	469	2·08	2·68	1·4	4·6

The effect of the administration of small quantities of 5-HTP was a small rise in the serotonin content of the tissues. The spleen is an extremely rich source of serotonin. Several interesting facets of serotonin metabolism are revealed by this work. The specific radioactivity of the serotonin is an index of the metabolic rate of serotonin in the tissue. Thus, kidney and liver are the most active sites, while the serotonin of the spleen is not in such an active state. It could be seen that the total radioactivity of the kidney is enhanced very much subsequent to the administration of LSD. This suggests that a considerably larger amount of the 5-HTP is transported to the kidney in the animal treated with LSD. The increase in the specific activity of serotonin is highest in the kidney, while on a percentage basis it is next highest to the brain tissues. This indicates an increased turnover of serotonin in the kidney and also a general increase in the serotonin content of the different parts of the viscera.

In the cerebrum there is no increase in the serotonin content in the animal treated with LSD. This is presumably due to the rapid destruction of the serotonin by oxidation, as is indicated by the higher specific activity of the serotonin. In other parts of the brain also, whereas the serotonin content increases only by approximately 40 per cent, the specific activity increases by about 350 per cent.

Thus these results indicate an overall increased metabolism of serotonin and an increase of the content of serotonin itself in all parts of the body examined, except in the cerebrum, subsequent to the administration of LSD. Whether the effects of LSD are specific or are shown by its non-hallucinogenic analogue, bromo lysergic acid diethylamide, and whether the action of LSD is limited to serotonin only without significant effects on other neurohormones such as noradrenaline is being investigated.

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Effect of the Bilateral Section of the Splanchnic Nerve on Erythropoiesis

Despite much recent work on erythropoietin which has established the important part played by the humoral factor in erythropoiesis, participation of the nervous system in the regulation of erythropoiesis has not been established. In view of the report of Jacobson *et al.*[1] suggesting the renal production of erythropoietin, as well as the work of Nakayama[2] that showed the effect of the bilateral section of splanchnic nerve in abolishing the decrease of renal blood-flow on loss of blood or anoxic anoxia, we performed a bilateral section of splanchnic nerve in rats to observe the effect on erythropoiesis.

The splanchnic nerve was sectioned bilaterally in the Wistar female rats (180–200 gm.) through the

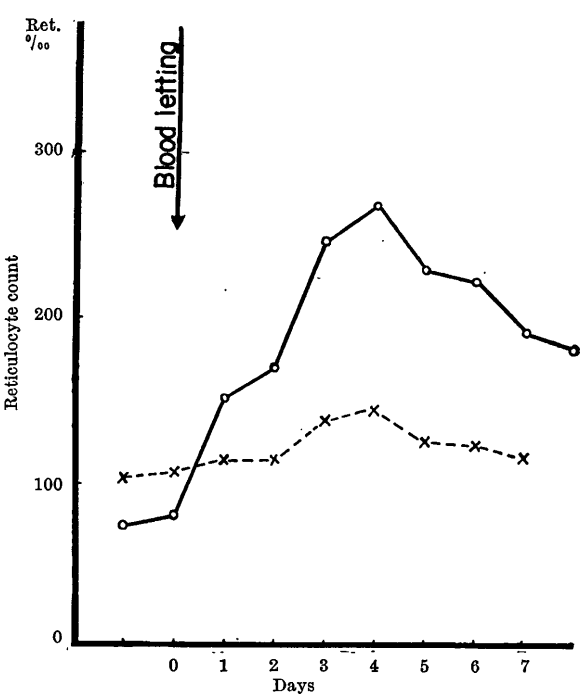

Fig. 1. Effect of bilateral section of splanchnic nerve on reticulocytosis after blood-letting (30 per cent of total blood volume). Each point represents the mean value for five rats. o, normal; ×, operated