indeed being removed since it was eluted after the major FSH fraction.

Our recommended procedure for preparing FSH free from LH and albumin is, therefore, an initial fractionation of *CP* 1 on 'Sephadex *G*-100' to remove inactive protein and subsequent fractionation and refractionation on DEAE-'Sephadex'. Recent work suggests that 'Biogel-150' may replace 'Sephadex *G*-100'. Two passages down DEAE-'Sephadex' are generally required to obtain the desired product. The latter columns particularly require to be closely scanned since the precise location of LH, FSH and albumin and the major ultra-violet absorption peak are somewhat variable, presumably due to formation of complexes between the various components. Recovery of activity on the columns as described here was 70–83 per cent.

A typical fractionation is shown in Fig. 1. Specific biological assays for FSH and LH demonstrated that the final product contained the equivalent of 1,000 (with 95 per cent fiducial limits of 730–1,430) mg first I.R.P.-human menopausal gonadotrophin (HMG) per ml. as FSH and less than 20 mg first I.R.P.-HMG per ml. as LH. (These results in I.U. are 143 (104–204) I.U. as FSH and < 10 I.U. as LH.)

Purified FSH showed no loss of activity when compared with a control after incubation at 4° C for 2 weeks in 0·05 M cysteine, 0·05 M mercaptoethanol and 0·5 per cent serum albumin. Treatment with 0·05 M sodium nitrite in acetate buffer *p*H 5·0 for 43 h at 4° C caused 85 per cent loss of FSH activity, 64 per cent of which was recoverable after dialysis against 0·1 M mercaptoethanol (35 vol.) for 20 h, indicative of oxidation of –SH to –S–S– by sodium nitrite and reduction back to the original –SH groups by mercaptoethanol. Equal portions of FSH which had been dialysed for 24 h at 4° C against 8 M urea in acetate buffer *p*H 5·0 retained 50 per cent of the original activity, but this was completely lost by alkylation of the free SH groups with iodoacetamide (3·3 per cent; *p*H 7·0) for 4 h at 4° C. Treatment of FSH in formic acid (90 per cent; 1 ml.) at −10° C for 2·5 h with performic acid (0·5 ml.) prepared from a mixture of formic acid (90 per cent; 9·5 ml.) and hydrogen peroxide (30 per cent w/v; 0·5 ml.) caused complete loss of activity, but some 50 per cent of the activity was lost on treatment with formic acid (90 per cent) alone at −10° C for 5 h. FSH activity was found to be labile to acid and treatment with 0·2 M citrate buffer (*p*H 2·2) at 37° C for 20 h or with 0·05 M ascorbic acid (*p*H 2·0) at 4° C for 2 weeks caused complete loss of activity. This is probably associated with the cleavage of *N*-acetylneuraminic acid residues since treatment of FSH with *Vibrio cholerae* neuraminidase at *p*H 5·6 at 37° C for 2 h also caused complete loss of activity. No loss of activity was encountered on treatment of FSH with chymotrypsin at 37° C for 13 or 24 h in 0·2 M phosphate buffer (*p*H 7·0) and attack by trypsin was relatively slow. No loss of activity was encountered at 3–4 h incubation but after 16 h only 16 per cent activity remained. Using five times the concentration of trypsin some 50–60 per cent activity still remained after 8 h, but there was complete loss after 25 h. Pronase caused complete loss of activity after only 2 h incubation at *p*H 7·0 and 34° C and was by far the most destructive of the proteolytic enzymes tested.

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REGIONAL LOCALIZATION OF LYSERGIC ACID DIETHYLAMIDE IN MONKEY BRAIN

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THE hallucinogenic agent, *d*-lysergic acid diethylamide (LSD), profoundly alters integrative aspects of perception. Emotional functioning is also markedly affected and there is considerable evidence of central sympathetic discharge. On the other hand, the discrimination of primary modalities of sensation including touch, posture and kinaesthesia is not disturbed nor is voluntary movement impaired[1].

Neurophysiological investigations have revealed an impaired function in local regions of the brain after LSD administration. Thus, there is a highly specific depression of synaptic transmission in the lateral geniculate body of the cat[2]. In human subjects with subcortically implanted electrodes, LSD causes increased activation in the limbic system which is not present in simultaneously recorded neocortical leads[3].

The characteristic effects of LSD in man are produced after minute doses (less than 1 μg/kg) and persist for 8–12 h. Recently, it has been shown that after the administration of 2 μg/kg LSD intravenously to human subjects, the drug disappears slowly from plasma with a half-life of 3 h, and measurable quantities persist for 8 h (ref. 4). Moreover, there is an excellent correlation between the rate of disappearance of LSD from the plasma and the recovery of a normal performance on a simple arithmetic test. It would thus appear that impairment of mental functioning may be related to a continued presence of the unchanged drug.

In the present investigation we have examined the hypothesis that the effects of LSD on specific areas of mental functioning might be related to a capacity of the drug to selectively concentrate in specific regions of the brain. The regional localization of LSD in the brain of the squirrel monkey has been studied using a sensitive and specific fluorometric method for the measurement of unchanged LSD. The subcellular localization of LSD in several areas of the brain has also been examined.

Four adult squirrel monkeys (*Saimiri sciureus*) were used in these investigations. In one animal, which received 2 mg/kg LSD, the sub-cellular distribution of LSD in various regions of the brain was determined, while in the other three, which respectively received 2, 1 and 0·5 mg/kg of LSD, the regional distribution of LSD in the brain was investigated. LSD (Sandoz) was administered to the conscious animal by a slow intravenous injection over a 10-min period. The dose for each monkey was dissolved in 0·6 ml. saline. Twenty minutes after the end of the infusion the animal was killed by an intracardiac injection of air. The brain was perfused *in situ* with 250 ml. saline by way of the ascending aorta in order to remove as much blood as possible from the brain tissue. The brain was then removed and frozen

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at −25° C in an acetone–dry ice mixture after which it was cut coronally into 3-mm sections using a brain macrotome. Using the atlas of Gergen and MacLean[5], each section was dissected into discrete regions which were kept in ice until analysed.

The LSD content of the dissected areas of brain was determined by the spectrophotofluorometric technique of Axelrod *et al.*[4], which is specific for LSD. As little as 0·001 μg per ml. of LSD can be measured by this method. The mean difference of duplicate determinations was 7·8 ± 2·4 per cent of the values.

The subcellular localization of LSD in various areas of the brain was examined by a method which will be described in detail elsewhere[7]. Tissues were gently homogenized in 10 ml. 0·25 M sucrose with a 'Teflon' pestle. The homogenate was centrifuged at 53,000*g* for 1 h in a discontinuous sucrose gradient prepared by successively placing into a *SW* 25 lusteroid tube 10 ml. 1·17 M sucrose, 10 ml. 0·73 M sucrose, and 10 ml. of the brain homogenate. In this technique the pinched-off nerve endings (synaptosomes) and mitochondria are located at the interface of the 1·17 M and 0·73 M sucrose layers, and the microsomal–myelin layer appears at the interface of the 0·25 M and 0·73 M sucrose layers. These layers, as well as the supernatant fluid, were removed with a Pasteur pipette and assayed for LSD content.

LSD was unequally distributed in the different areas of the brain (Table 1). The highest concentrations of the drug were found in the pituitary and pineal glands. These concentrations were 7–8 times the amount found in the cortex. The anterior pituitary had approximately twice the concentration found in the posterior pituitary gland. Structures of the limbic system, that is, the hippocampus, amygdala, fornix, and septal region, contained 2–3 times the cortical concentration of LSD. Among the deep cerebral structures LSD was 2–5 times more concentrated in the visual and auditory areas and hypothalamus than in the cortex. The extrapyramidal system and thalamus contained approximately 1·5 times the cortical concentration. Among the superficial cerebral structures LSD was about equally distributed between white and grey matter. LSD was not notably concentrated in the visual cortex. The brain stem contained concentrations of LSD similar to that found in the cortex. In one experiment the blood concentration of LSD was determined just prior to killing the animal and was found to be the same as the cortical concentration. The cerebellum contained significantly less LSD than the cerebral cortex. In one animal the concentration of LSD in the iris was measured and found to be 18 times as high as that of the cortex.

Table 1. Localization of LSD in Different Areas of Monkey Brain

	Exp. 1	Exp. 2	Exp. 3	Mean ± *S.E.*
Superficial cerebral structures				
Frontal grey	1·00	1·00	1·00	1·00 ± 0
Frontal white	1·60	1·78	1·24	1·54 ± 0·16
Frontal grey and white	1·30	1·39	—	1·35 ± 0·05
Temporal grey	1·33	—	0·84	1·09 ± 0·25
Parietal grey	0·80	1·22	1·12	1·05 ± 0·13
Parietal white	0·65	0·83	1·18	0·89 ± 0·16
Corpus callosum	1·20	0·56	1·18	0·98 ± 0·21
Visual cortex	0·63	1·28	1·42	1·11 ± 0·24
Extrapyramidal system				
Caudate	1·22	1·39	1·08	1·53 ± 0·23
Putamen	1·47	1·39	1·40	1·42 ± 0·03
Globus pallidus	2·27	1·61	1·84	1·91 ± 0·19
Substantia nigra	1·73	1·44	—	1·59 ± 0·15
Visual and auditory reflex areas				
Optic chiasma	4·72	6·66	5·00	5·40 ± 0·61
Lateral geniculate	3·16	2·39	3·80	3·12 ± 0·41
Superior colliculus	3·17	2·66	3·42	3·08 ± 0·22
Periaqueductal grey	2·13	2·66	—	2·39 ± 0·26
Medial geniculate	—	2·61	4·30	3·45 ± 0·84
Inferior colliculus	1·86	2·39	2·60	2·35 ± 0·26
Hypothalamus				
Anterior	2·19	3·06	2·44	2·56 ± 0·26
Posterior	3·51	2·83	3·70	3·34 ± 0·26
Limbic system				
Amygdala	1·70	2·28	3·40	2·40 ± 0·48
Hippocampus	2·22	1·80	2·80	2·30 ± 0·26
Fornix	3·00	2·66	3·56	3·07 ± 0·26
Anterior commissure	3·05	3·44	—	3·70 ± 0·26
Septum	2·11	—	2·44	2·27 ± 0·16
Cerebellum				
Cortex	0·66	0·83	0·94	0·81 ± 0·09
Dentate nucleus	0·77	—	—	0·77
Pituitary gland				
Anterior	6·00	11·10	14·45	10·51 ± 2·45
Posterior	3·40	6·66	6·70	5·58 ± 1·09
Pineal gland	7·00	5·55	8·00	6·85 ± 0·71
Brain stem				
Midbrain	0·59	1·44	1·50	1·17 ± 0·29
Pons	0·73	1·44	1·00	1·05 ± 0·21
Medulla	0·91	1·28	0·94	1·04 ± 0·12
Thalamus				
Anterior	1·59	2·78	2·20	2·19 ± 0·34
Posterior	1·30	0·50	1·38	1·06 ± 0·28
Blood	—	—	1·06	
Iris	—	—	18·3	

The amounts of LSD administered were 2 mg/kg in exp. No. 1, 1 mg/kg in exp. No. 2, and 0·5 mg/kg in exp. No. 3. LSD concentrations are expressed as ratio to frontal grey concentration which was 0·94 μg/g in exp. No. 1, 0·30 μg/g in exp. No. 2 and 0·14 μg/g in exp. No. 3.

Table 2. Comparison of LSD Content of Various Regions of the Brain

	Ratio to frontal grey* concentration	*P* value
Superficial cerebral structures	1·12 ± 0·03	—
Extrapyramidal system	1·69 ± 0·09†	< 0·001
Visual and auditory reflex areas	3·37 ± 0·20†	< 0·001
Hypothalamus	2·96 ± 0·00†	< 0·001
Thalamus	1·46 ± 0·10†	< 0·05
Limbic system	2·74 ± 0·15†	< 0·001
Pituitary gland	8·05 ± 1·75†	< 0·02
Pineal gland	6·85 ± 0·71†	< 0·005
Cerebellum	0·81 ± 0·09†	< 0·02
Brain stem	1·09 ± 0·19	> 0·8

* Mean ± *S.E.M.*
† Significantly different from superficial cerebral structures (Student's *t* test).

Table 3. Subcellular Distribution of LSD in Monkey Brain
Percentage of total LSD in subcellular fractions

	Supernatant	Microsomes-myelin	Synaptosomes-mitochondria
Hippocampus	75	20	5
Frontal grey	73	19	8
Frontal white	77	20	3
LSD added to homogenate	79	15	6

Sub-cellular fractions of the hippocampus, frontal grey matter, and frontal white matter were prepared as already described. In a separate experiment, LSD was added at 4° C to an intact brain, which was then homogenized and carried through the previously described isolation procedure. In the areas of the brain examined, LSD was largely confined to the soluble supernatant fluid (Table 3). The sub-cellular localization was the same when LSD was added to the homogenate. These results would indicate that LSD does not localize in a specific subcellular brain particle.

After intravenous administration of LSD, the drug is unequally distributed in the different areas of the brain of the monkey, with about a ten-fold variation between structures with the highest and lowest concentrations. These differences in concentration are probably not due to differences in regional blood flow to these areas since these investigations were performed 30 min after the start of the infusion. Although the initial distribution of LSD in the brain would be determined by the regional cerebral blood flow, after 30 min it might be expected that the various tissues would be approaching equilibrium with the blood levels of LSD and regional blood flow would no longer be the determining factor. This is supported by the fact that a comparison of regional blood flow values[8] and LSD concentration shows no significant correlation ($r = -0{\cdot}03$; $P > 0{\cdot}9$).

It is also unlikely that the unequal distribution of LSD is simply the result of its lipid solubility and consequent predilection for white matter. There was no apparent difference in the LSD content of cerebral cortex and white matter. Furthermore, LSD was highly concentrated in structures which are not especially rich in white matter such as the pituitary and hypothalamus.

Another factor which should be considered is the vascularity of different regions of the brain. However, since the maximum vascularity in the brain[8] is of the order of 3 per cent and since the blood concentration of LSD was the same as that in the cortex, the different blood content of various regions of the brain could not account for the

magnitude of the differences in regional LSD concentration found. Furthermore, attempts were made to wash the blood from the brain before the determinations were made.

In an investigation of the regional distribution of mescaline-^{14}C in the cat brain, Neff *et al.*[10] found that mescaline, like LSD, was present in high concentration in the pituitary and lateral and medial geniculate bodies. In contrast to the present results with LSD, high concentrations of mescaline were found after 30 min in the cerebrocortical grey matter while white matter contained only 25 per cent as much mescaline.

Arnold *et al.*[11] investigated the distribution of ^{14}C-LSD in the mouse brain by an autoradiographic technique. They also divided the brain into cerebrum, brain stem, medulla, and cerebellum, and determined the total radioactivity in these regions. It was found that cellular areas had higher activity than white matter and that these areas could be arranged in order of decreasing activity as follows: limbic lobe, hippocampus, basal ganglia, grey matter around the third ventricle, cerebral cortex and cerebellum. As the authors point out, these studies do not distinguish between LSD and its metabolites. However, in the regions they analysed, the order of decreasing activity is essentially the same as found in the present investigation.

LSD produces marked perceptual distortions. These effects appear to be the result of affectively determined perceptual alterations and hallucinations. There is, however, no marked impairment of discrimination of the primary modalities of sensation[4]. In the present investigation, there was no particular localization of LSD in the primary sensory areas of the cerebral cortex. The drug, however, was highly concentrated in subcortical visual centres, which have been implicated in the production of visual hallucinations[12]. LSD was also found in high concentrations in the limbic system and in the hypothalamus, areas which are thought to be involved in the mediation of affective behaviour[13]. Monroe *et al.*[5] found that the most significant response to LSD was the appearance of subcortical paroxysmal activity, which was most often limited to the septal, anterior hypothalamic, or amygdaloid-hippocampal regions. Dramatic behavioural changes were usually associated with the electroencephalographic changes in these areas. Certain mental effects of LSD have previously been shown to be related to persistent plasma levels of unchanged LSD[6]. It is possible that some of the effects of LSD on 'emotionality' and affectively determined perceptual alterations might be related to the selective concentration of this drug in areas thought to be involved in these functions.

LSD produces central sympathetic stimulation with effects which include hyperglycemia, mydriasis, pilo-erection, tachycardia, leucocytosis, and a rise in body temperature. These effects are related to the stimulation of mesencephalic and diencephalic structures[14], areas which selectively concentrate LSD.

The above evidence is merely suggestive and obviously no conclusions can be drawn from this investigation as to the mode of action of LSD in producing its characteristic effects. Furthermore, in order to obtain adequate tissue levels for fluorometric assay relatively high doses of LSD were administered in the present study. It is possible that with smaller doses the relative concentration of LSD in those areas which we found had high concentrations might be more pronounced.

In the investigations of subcellular distribution, the localization of LSD in the cell sap would suggest that LSD is not bound to subcellular particles. Arnold *et al.*[11], on the basis of their autoradiographic investigations, concluded that the activity was present in the cytoplasm and not in the nucleus.

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INCREASED RED CELL BREAKDOWN IN ADOPTIVELY IMMUNIZED YOUNG *NZB* MICE

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MICE of the *NZB* strain spontaneously develop haemolytic anaemia with autoantibodies against red cells[1]. Holmes, Gorrie and Burnet have described a positive direct Coombs's test (D.C.T.) in such mice, and have been able to detect this antibody in young mice adoptively immunized with spleen cells from older D.C.T. positive animals[2]. We recently described an anti-human red cell cross-reacting antibody (A.H.R.) in these mice[3], and now, using the spleen cell adoptive immunization technique, have been able to induce this antibody in young animals. Using red cells labelled with chromium-51, we have tried to relate the presence of these two antibodies to increased red cell breakdown which, in man, is an essential feature of autoimmune haemolytic anaemia.

Animals. The *NZB* mice were part of the colony kept in conventional animal housing at Taplow. Donors were selected on the basis of sex and their antibody status. Recipients, 4–6 weeks of age, were separated into three groups prior to the injection of spleen cells (Table 1): those receiving spleen cells from donors which were (*A*) A.H.R. + D.C.T. +, (*B*) A.H.R. − D.C.T. +, (*C*) A.H.R. − D.C.T. −. Each group contained both males and females, individual mice receiving cells from a donor of the same sex.

Spleen cell suspensions. In each group different numbers of donor animals were killed and their spleens taken after perfusion with saline. Spleen cell suspensions were prepared by gentle homogenization of individual spleens in a

Table 1. Groups of Young *NZB* Mice receiving Spleen Cells from *NZB* Donors

Group	Donor antibody status	No. of recipients Male	Female
A	D.C.T. + A.H.R. +	14	8
B	D.C.T. + A.H.R. −	6	15
C	D.C.T. − A.H.R. −	12	11

D.C.T., direct Coombs's test (+ or −).
A.H.R., antibody against human red cell antigen present in serum (+ or −).