following: 19 of 31 (61%) were negative in the treated group (2 were toxic and could not be tested). In the untreated group, 17 of 33 (51%) were negative. Although in the treated group both the number of mice having virus in the blood, and the amounts of virus as determined by titration, were slightly less than in the untreated controls, the differences were not nearly so marked as those found in the brains. Of the treated group, the brains of 23 of 33 animals, or approximately 70%, were negative in contrast to the untreated, in which 33 of 55, or 45.5%, were negative. However, animals of both groups, when paralyzed, showed no significant difference in the amount of virus present in the brains.

Discussion. The effects of treatment with 2,6-diaminopurine of mice infected with Russian Spring-Summer Encephalitis virus have been investigated. The chemical, a substance which is involved in nucleic acid synthesis, has been shown(9) to have an effect on the proliferation of the virus in tissue culture. It has now been demonstrated that DAP exerts a therapeutic effect on animals infected with RSSE even if treatment is begun 24 hours after the virus has been inoculated.

The mechanism of action of the DAP is unknown, but the studies on the distribution of the virus in brain and blood of the treated and untreated animal gives us a clue as to the point of action of the substance.

Since as much virus is present in the blood of the treated animals as the untreated, DAP apparently does not have an effect on the invasiveness of the virus. When the virus is inoculated intracerebrally, the DAP is ineffective, and treated animals which are not protected by the DAP have as much virus in their brains as do the untreated controls. Hence the DAP exerts its activity either on the "blood-brain" barrier or directly on the cells of the brain.

Summary. The compound 2,6-diaminopurine when administered in doses of 70 mg/kg caused an increase in the survival rates of mice infected with Russian Spring-Summer Encephalitis by the intraperitoneal route. It was effective when treatment was started 24 hours after the inoculation of the virus.

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Metabolism of Amines. II: Mitochondrial Localization of Monoamine Oxidase. (19006)

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Despite numerous investigations of its enzymatic function, monoamine oxidase has not been significantly purified. As with other systems that are bound to insoluble particles, the difficulty lies in the extraction of sufficient amounts of active protein for the application of classical methods of fractionation. On the other hand, this difficulty implies a degree of association with organized structures that deserves investigation and suggests that the chemical neighborhood of the enzyme should be explored with fractionations that preserve the integrity of cytological units.

Previous investigators, notably Claude, Hogeboom, Schneider, Dounce, and their collaborators, have recognized the power of morphological fractionation. (See Schneider and Hogeboom(1) for review). In the present investigation it remained to apply their methods and correlate with their results.

Procedure. Adult male rats (170-230 g) of a Sherman strain were used in all experiments. After a week of observation with an unrestricted diet of Purina fox chow, each animal was allowed water during a final period

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of 18 hours and then was sacrificed by decapitation. The liver was removed immediately and taken into a cold room (0.-3°C) for centrifugal fractionation in 0.88 M sucrose, according to the method of Hogeboom *et al.*(2). As a preliminary step, the liver was passed through an Altmann press to remove connective tissue. About 3.0 g of the pulp were weighed quickly into a Potter homogenizer tube(3), sucrose solution was added to a final volume of 30.0 ml, the preparation was homogenized for about 5 minutes with a motor driven pestle, and then centrifuged. At each stage of the fractionation process, indicated in Fig. 1, the supernate was separated from the precipitate by decantation, fresh sucrose was added to the precipitate and the latter again was dispersed by a motor driven lucite paddle. Since the amount of sedimentation effected is determined by the product of centrifugal force and time, within wide limits of variation of either factor, it was convenient to plan the work in terms of "centrifugal units" (relative centrifugal force × time in minutes × 10^3)(4). In terms of these units the components of the homogenate were separated into three classes: (1) those that were sedimented consistently by 6 units (fraction N), (2) those that remained suspended after 6 but sedimented with 400 units (fraction M, and the fraction NW which had been swept down once with the larger particles but on redispersion was indistinguishable from M), (3) the supernate (S) consisting of fine particles and soluble material, which remained suspended during repeated centrifugations of 400 units.

The activity of monoamine oxidase in the original homogenate, and in each of four final fractions (N, M, NW, S), was determined by the rate of ammonia release from tyramine(5). Nitrogen was determined in the

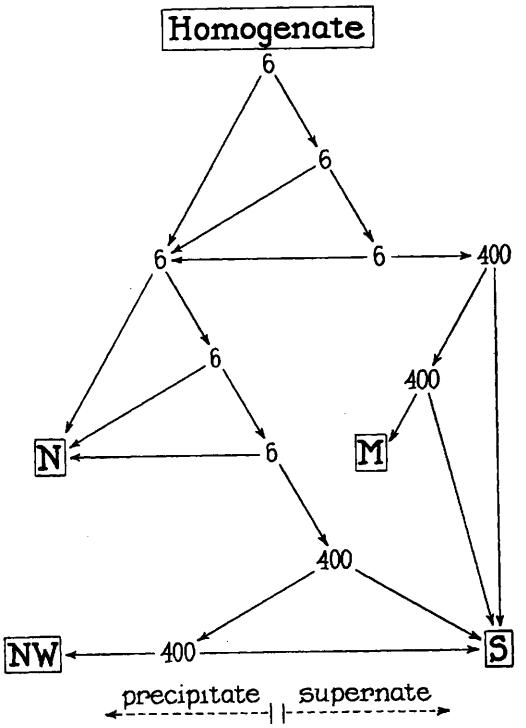

FIG. 1. Diagram of the centrifugal fractionations in 0.88 M sucrose. The numbers at each junction indicate the centrifugal units (1000 G × min)(4) that were applied to a suspension; the emergent arrows trace the fate of the separated portions, sediment to the left and supernate to the right. Fractions that were analyzed are enclosed in squares.

earlier experiments by the microdiffusion method of Conway(6) and later by direct nesslerization, as applied by Eder(7) to the mercury-catalyzed digestion mixture of Hiller *et al.*(8). An experiment was considered satisfactory if the enzymatic activity and the nitrogen content of the original homogenate were accurately recovered in the summation of values of derived fractions (Table I). Because of the invariable occurrence of intact cells and cytoplasmic fragments in the nuclear fraction (N), it was not possible with this method to determine whether or not enzymatic activity was contained in the nucleus

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6. Conway, E. J., Microdiffusion Analysis and Volumetric Error, London, 2nd edition, 1947.

7. Eder, H. A., personal communication.

8. Hiller, A., Plazin, J., Van Slyke, D. D., *J. Biol. Chem.*, 1948, v176, 1401.

TABLE I. Distribution of Monoamine Oxidase.

Fraction	Enzyme: Units g wet tissue	Enzyme: % of homogenate	Nitrogen: mM N g wet tissue	Nitrogen: % of homogenate	Specific activity: Units enzyme / mM N	Specific activity: % of homogenate
N	5.3 ± 1.6	21.9	.49 ± .18	23.1	11.7 ± 3.1	98 ± 17
NW	3.1 ± 1.5	12.9	.11 ± .06	5.2	33 ± 18	312 ± 218
M	13.7 ± 1.8	56.8	.56 ± .15	26.4	26 ± 6.9	219 ± 25
S	1.9 ± 1.2	7.9	.91 ± .33	42.9	2.6 ± 2	20 ± 14
Sum	23.8	99.5	2.07	97.6		
Homogen.	24.1 ± 2		2.12 ± .38		11.9 ± 2.6	

The fractions are those obtained by differential centrifugation in 0.88M sucrose(2). The numbers are the means and stand. dev. from 13 exp.

in addition to its predominant localization in the adherent mitochondria. To test this point attempts were made to purify the nuclei contained in this fraction. Rehomogenization at different acidities and differential centrifugation in 0.88 M sucrose yielded preparations that were inferior to those obtained by the citric acid method of Dounce(9); the latter, therefore, was employed for the study of nuclei free from detectable cytoplasm. Every analyzed fraction was examined microscopically, both in the fresh state with a phase contrast microscope and after fixation with 4% osmic acid and staining with hematoxylin, Giemsa or Wright's stain.

Observations. It may be seen in Table I, which summarizes 13 concordant experiments, that the enzymatic activity became concentrated in the mitochondrial fractions, M and NW. The specific activity (units of enzymatic activity per unit of nitrogen) in fraction M averaged 219% of the specific activity of the original homogenate. The nuclear washing fraction NW was histologically identical to fraction N; when the activities contained in these two fractions were summed it was found that from 66 to 75% of the total activity had been recovered with isolated mitochondria.

The remainder of the original activity passed into the fraction N, which consisted of nuclei with variable appendages of cytoplasm, unbroken cells and small amounts of connective tissue fragments and red blood cells. Separate experiments, in which negligible specific activities were found with blood, subcutaneous connective tissue and choroid plexus, made it unlikely that connective tissue or vascular elements in the fraction contributed to any important extent. Similarly, it was found in 4 experiments that purified nuclei, prepared by the citric acid method, lacked appreciable enzymatic activity.

Upon histological examination of the different fractions, it was found that those prepared in 0.88 M sucrose retained the normal structure and staining properties of the formed elements, including the nuclei of fraction N. The cytoplasm-free nuclei, obtained after exposure to citric acid, were markedly altered, however; phase microscopy revealed in them opaque strands which were lacking in fresh liver nuclei, and in the fixed material it was seen that they had suffered a considerable loss in their capacity to stain with hematoxylin and with methylene blue.

Discussion. The data indicate that the enzymatic activity is predominantly associated with the mitochondria, and suggest that it might be confined to these elements. If it be assumed that the activity which passed into fraction N was carried by the numerous mitochondria in the cytoplasmic fragments it is possible to explain why the specific activity of this fraction remained the same as that of the original homogenate: both mitochondria and the inactive, protein-rich supernate (fraction S) had been removed in equivalent amounts. On the other hand, if the nuclei had a moderate or high specific activity in addition to that contributed by the mitochondria, it might be expected that the resultant specific activity of the fraction would have been greater than that of the original homogenate. The absence

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of significant enzymatic activity from the cytoplasm-free nuclei also favors the interpretation that the locus is exclusively mitochondrial, but it must be conceded that these nuclei could have suffered extraction of enzyme in the course of purification.

It is of interest to note that the mitochondrial localization of monoamine oxidase might have been expected from the precedent of other systems which transfer electrons to oxygen: d-amino acid oxidase(10), uricase (11), and processes dependent upon cytocrome oxidase(12). This convergence of oxidative systems suggests that the mitochondrion is a favorable location for the reduction of oxygen; whether or not there is a more intimate functional association between these processes and an enzyme that deaminates potent pharmacological agents is a question that awaits future investigation.

Summary. (1) The monoamine oxidase activity of rat liver cells is localized predominantly, and perhaps exclusively, in the mitochondria. (2) In this respect it conforms to a precedent since other aerobic processes have been shown to have a mitochondrial localization.

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Streptococcal Bacteriostatic Antibody in Patients Treated with Penicillin.* (19007)

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The antibacterial activity of the serum of patients infected with the beta-hemolytic *Streptococcus* has been studied by a number of investigators. Bacteriotropins for this organism were first demonstrated by Hare(1). Kuttner and Lenert(2) noted that the bacteriostatic antibody was type-specific, persisted for many months, and was unrelated to the development of antistreptolysin O. From a detailed study of 3 patients, Rothbard(3) concluded that this immune substance first appeared in the third to fifth week following infection, was present for at least 37 weeks and was type specific. A higher incidence of bacteriostatic antibody in rheumatic fever patients than in those with uncomplicated streptococcal infections was observed by Rothbard, Watson, Swift, and Wilson(4). The close association between the presence of type-specific nucleoprotein (M) in the infecting *Streptococcus* and the later development of antibacterial immunity has been recognized(5-7).

The administration of penicillin to patients with acute streptococcal pharyngitis suppresses the formation of antistreptolysin O and anti-streptokinase(8-12). The work re-

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