This sedimentation rate is in accord with that reported by Muus (*2*) for her crystalline human salivary amylase. She reported a sedimentation rate (S_{20}) of 4.6 Svedberg units in a preliminary experiment. Neither of the afore-mentioned values has been corrected to zero concentration.

In this work, α-amylase has been located in both the electrophoretic and ultracentrifugal patterns of human parotid gland secretion. It has an average electrophoretic mobility of -1.4×10^{-5} cm²/volt-sec and a sedimentation rate ($S_{20,\,w}$) of 4.1 Svedberg units in the Miller-Golder buffer of 0.1 ionic strength and *p*H 8.5.

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Possible Interrelationship between Release of Brain Norepinephrine and Serotonin by Reserpine

The finding of a solvent system for the extraction of catechol amines has permitted the development of a simple fluorimetric method for the rapid determination of norepinephrine in various body tissues (*1*). The catechol amine extracted from rabbit brain was predominantly norepinephrine, with a small proportion of epinephrine, as shown by activation and fluorescence spectra (*2*), by rate of oxidation at various *p*H values, and by paper chromatography. Its distribution in brain was found to be in general like that of serotonin; that is, it is high in brain stem and low in cortical areas and cerebellum, as noted by Vogt, who used a bioassay procedure (*3*).

In previous studies, we have shown that reserpine impairs the capacity of body tissues to maintain serotonin in a bound form (*4*), thereby causing its liberation from brain and other tissues

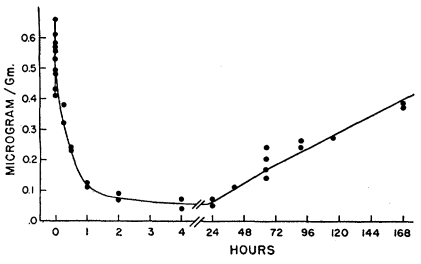

Fig. 1. Norepinephrine content of rabbit brain stem at various times after intravenous administration of reserpine (5 mg/kg).

(*5*). Recent observations that reserpine also lowers the content of catechol amines in various body tissues (*6*) prompted an investigation concerning the nature of this phenomenon and its possible relationship to the release of serotonin.

Rabbits received reserpine (5 mg/kg) by intravenous injection and were sacrificed at various times thereafter. The brains were removed immediately, and the brain stems were analyzed for norepinephrine. The level of norepinephrine declined rapidly, a definite drop occurring within 15 minutes and about 90 percent disappearing within 4 hours. The level remained low for about 48 hours and then increased slowly, attaining the normal value after about 7 days (Fig. 1). The shape and duration of the curve is practically identical with that depicting the effect of reserpine on serotonin in brain (*7*).

The effect of various doses of reserpine on the norepinephrine content of the brain was determined. Doses as low as 0.1 mg/kg had a definite effect, and 1.0 mg/kg was as effective as 5 mg/kg (Fig. 2). The dosage response curve was almost identical with that found for release of serotonin.

It has been shown that reserpine appears to exert an irreversible action, disappearing rapidly from brain and other tissues and not appearing to act through a metabolic product (*5*). Accordingly, the depletion of norepinephrine as well as of serotonin persists long after reserpine has virtually disappeared. The almost identical patterns of effect on serotonin and norepinephrine both with regard to duration and with regard to response to various doses of reserpine suggest that the release of the biogenic substances in brain are closely linked phenomena. It is possible that norepinephrine and serotonin are normally bound in brain tissue by similar mechanisms which are impaired by reserpine. Another possibility is that the serotonin released by reserpine in turn releases norepinephrine (or vice versa).

The release of catechol amines from the adrenal glands was also measured. Depletion of the amines occurred over a period of 16 hours after administration of reserpine (5 mg/kg), and was virtually complete for a period of several hours. The content of the adrenal amines then gradually rose and achieved the normal value after about 7 days, a time when brain norepinephrine and serotonin were again normal. The loss of medullary amines did not result from a direct action of reserpine, since it was prevented by spinal transection at T_1, indicating, as do the findings of Holzbauer and Vogt (*6*), that the depletion was the result of stimulation of sympathetic centers in brain; this suggests that the depletion might be related to norepinephrine (or serotonin) changes in the central nervous system. It is pertinent that reserpine, which produces a generalized parasympathetic predominance, induces a prolonged stimulation of the sympathetic nerve innervating the adrenal gland.

Peripheral norepinephrine was also depleted following administration of reserpine, doses as small as 0.1 mg/kg lowering the content of amine in the heart by about 85 percent within a period of 4 hours. Reserpine also induced the depletion of norepinephrine from spleen. Section of the cord at C_7 or at C_2 did not prevent the release of norepinephrine from the heart following doses of 5 mg/kg of reserpine, indicating that with these doses, the liberation resulted mainly from the action of reserpine directly on the peripheral storage depots.

The ability of reserpine to release norepinephrine and serotonin throughout the body indicates that changes in the amines centrally as well as peripherally must be considered in explaining the over-all action of the *Rauwolfia* alkaloid. Accordingly, the extent to which changes in autonomic balance following administration of reserpine may be attributed to alterations of peripheral and brain norepinephrine is now under investigation. The emerging picture of reserpine as a releaser of serotonin, norepinephrine,

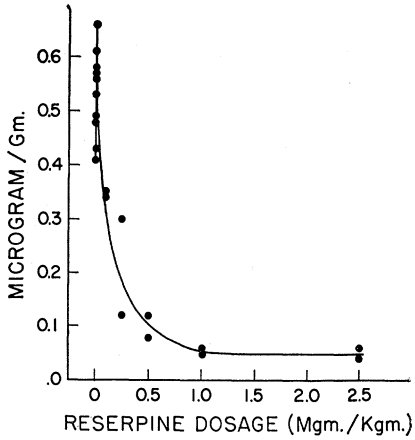

Fig. 2. Norepinephrine content of rabbit brain stem 4 hours after administration of various intravenous doses of reserpine.

and epinephrine throughout the body supplies a potent tool for studying the role and interplay of these neurohormones in the brain as well as peripherally.

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Isolation of a Nutritional Variant from a Culture of Rabbit Fibroblasts

The results of earlier studies dealing with the cultural characteristics of rabbit fibroblasts derived from normal tissue indicated that the over-all nutritional requirements of newly established strains vary as a function of their age *in vitro* (*1*). The quantitative aspects of the data were consistent with the concept that either a limited number of cells in the original population (inoculum) were capable of continuous growth in the medium employed or that such cells appeared in the population as a result of mutation. The present experiments (*2*) were undertaken to obtain information regarding the fundamental question of whether nutritional variants are present in a given culture after prolonged serial cultivation *in vitro*. Rabbit fibroblasts, strain RM3-56, which had been propagated serially *in vitro* for almost 2 years were selected for study (*1, 3*). On the assumption that the proportion of nutritional variants in a particular population might be too small to be detected by examining the progeny of single cells selected at random from the population, it was considered expedient to establish conditions which favor the proliferation of variants by altering the composition of the medium.

Table 1. Isolation of a nutritional variant from a culture of RM3-56 fibroblasts.

No. of passages *in vitro*	No. of cells*	No. of colonies†
79	1.1×10^6	1
87	0.9×10^6	0
91	1.5×10^6	2

* Second passage in medium 73.
† Colonies containing cells capable of continuous proliferation in medium 73.

The following media were employed: medium 56 was 5 percent chick embryo extract, 10 percent normal horse serum, and 85 percent S18 (volume for volume); medium 73 was 2 percent dialyzed horse serum, 98 percent S16. Solutions S16 and S18 are of similar composition and contain amino acids, vitamins, salts, and glucose (*3*). The procedure employed for propagating RM3-56 serially in medium 56 and the methods used in quantitative experiments were described previously (*3*). The experiments were based on the observation that RM3-56 fibroblasts fail to proliferate in medium 73 after the first subculture. Under these conditions, the nonproliferating cells gradually degenerate over a period of from 3 to 4 weeks. The regular schedule of fluid replacements was continued, to permit the detection of any cells capable of proliferating in this medium. The results of three experiments performed with cells of different ages *in vitro* are summarized in Table 1.

In the first and third experiments, three colonies, containing from 50 to 100 healthy fibroblasts, were observed after 9 to 16 days (second passage in medium 73). These cells proliferated rapidly, whereas the remaining fibroblasts degenerated. In the second experiment, none of the cells appeared capable of proliferating in medium 73. The cells comprising one of the colonies were subsequently subcultured, and their progeny continue to proliferate after 40 passages in medium 73. This variant or subline is designated RM3-73, to indicate both the tissue from which the culture was originally derived and the medium in which the strain is propagated. Strain RM3-73 also differs from RM3-56 in that it proliferates at a somewhat slower rate in medium 56. On the other hand, strain RM3-73 is indistinguishable from RM3-56 by such criteria as morphology and susceptibility to infection with vaccinia virus.

Of particular significance is the fact that only a small fraction of the cells in RM3-56 cultures are of the 73-type. For example, the three colonies isolated in experiments (Table 1) that employed 3.5×10^6 cells contained a total of only 225 cells when first observed. The proportion of RM3-73 cells in a population of RM3-56, calculated on this basis, is a maximal value because the 73 cells were proliferating throughout the course of the experiments. It is apparent that, under conditions where the ratio of two nutritional types of cells is less than 1 to 10^4, cloning techniques (*4–6*) are impractical for detecting the minority type. The method of selection applied under conditions where a large proportion of the cells fail to proliferate provides a practical means for studying large populations of cells with respect to nutritional cell types. Furthermore, the presence of nonproliferating cells serves the same purpose, in permitting isolated cells to proliferate, as the addition of irradiated cells ("feeder layer") described by Puck and Marcus (*5*). The method is particularly suitable for studying the genetics of cell lines whose nutritional requirements have been partially defined. In these instances, by combining the selection method and cloning techniques, it becomes possible to study somatic cells with any of the required nutritional factors as genetic markers. It is anticipated that the availability of a broad spectrum of nutritional cell types not only will be of aid in understanding the physiology of cultured cells but will enhance the usefulness of the tissue-culture method in virology and in studies on malignancy.

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Histochemical Demonstration of Enzymes Separated by Zone Electrophoresis in Starch Gels

Zone electrophoresis in starch gels is a rapid, simple, dependable method of high-resolving power for separating complex mixtures of proteins. We have combined this method with histochemical techniques for locating and identifying enzymes in tissue sections in order to analyze the enzymatic composition of biological material (*1, 2*). The numerous esterases responsible for the high level of esterase activity in the mouse liver