We tested the specificity of the $R^{10}S$ effect by determining whether it would similarly enhance the aggregation of other embryonic cells dissociated from 10-day liver, 6-day heart, and 4-day limb buds. In no case was there any demonstrable effect of $R^{10}S$ on the size of the aggregates of these cells. A supernatant medium was prepared (according to the procedure for $R^{10}S$) from monolayer cultures of 10-day embryonic liver cells; it had no demonstrable effect on the aggregation of 10-day embryonic retina cells.

Dialysis of the supernatant medium was essential for demonstration of the specificity of its effect. Thus, nondialyzed supernatants from monolayer cultures of liver, limb buds, heart, and retina cells enhanced the aggregation of 10-day embryonic liver cells. This nonspecific effect was found to be due to a serum component taken up by the cells during the first 48 hours in monolayer culture; it is then released into the serum-free medium during the 24-hour period preceding collection of the supernatant fluid. Dialysis removes or inactivates it.

Initial studies on the formation of the active constituent in $R^{10}S$ indicate that it does not accumulate in the culture medium if the monolayer cultures are kept at 4°C or are treated with cycloheximide (2 μg/ml) during the 24 hours preceding collection of the supernatant. This finding suggests that the formation or release of the active constituent by the cells requires biosynthetic processes involving protein synthesis. In this context it is of interest that low temperatures and inhibitors of protein synthesis also inhibit aggregation of embryonic cells dissociated with trypsin, presumably by interfering with the synthesis of constituents degraded by trypsin and required for continued aggregation of cells (*5*, *6*).

The enhancement of aggregation by $R^{10}S$ requires temperature-dependent processes in the responding cells. Thus, in cultures rotated for 24 hours at 4°C there is no effect; however, when these cultures are transferred to 38°C and further rotated for 24 hours, enhancement of aggregation occurs. Although at 4°C $R^{10}S$ had no visible effect, the active constituent was taken up by the cells. This was determined as follows. Dissociated 10-day retina cells dispersed in medium with $R^{10}S$ were maintained in rotation cultures at 4°C for 24 hours. The medium was then separated from the cells and tested on freshly dissociated 10-day retina cells at 38°C to determine whether it retained its typical activity. No measurable activity could be detected; this, together with the other results, strongly indicates that at 4°C the active constituent is taken up by the cells, but that its function in enhancing aggregation requires metabolic activity. Further evidence along this line is provided by the fact that if $R^{10}S$ is exposed at 4°C for 24 hours to 10-day embryonic liver cells, it does not appreciably lose its capacity to enhance the aggregation of retina cells at 38°C; this indicates that the $R^{10}S$ constituent is not taken up by liver cells to the same extent as it is by retina cells and further demonstrates its functional specificity. Thus, the enhancement of aggregation by $R^{10}S$ appears to involve two main phases, as suggested on the basis of earlier work (*5*): selective uptake and functional utilization. The mechanisms involved in these phases are being studied.

The active constituent of $R^{10}S$ is not destroyed by deoxyribonuclease or ribonuclease; it sediments by centrifugation overnight at 105,000*g*. This finding suggests that the active constituent has a particulate nature, as in the case of the factor which promotes aggregation of sponge cells (*4*). Our results raise questions concerning the characteristics of the active constituent in $R^{10}S$ and possible changes in its properties with cell differentiation; further studies should also determine whether the procedures used in its preparation and bioassay are effectively applicable to the isolation from other cells of materials which promote aggregation, or whether in other cases such materials are more strongly bound to the cells and not as readily obtainable in solution.

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Hematuria Following Administration of Ethanol

Abstract. *Hematuria, with some erythrocytes also appearing in the urine, was observed in male albino rats given 20 percent and 40 percent ethanol in lieu of drinking water for several weeks. However, the amounts of hemoglobin in the blood of the treated group did not change relative to those of the control group; this finding indicates that the degree of hematuria was not sufficient to produce an anemia.*

Although there have been numerous reports (*1*) describing the occurrence of hematuria in various pathological states, we are the first, to our knowledge, to describe hematuria following the administration of ethanol to an experimental animal.

The hematuria was first observed fortuitously after 15 weeks of administration of ethanol to experimental (male) Sprague-Dawley albino rats. The urine samples were observed to be darker in color than those of the control group given water. With respect to the amounts of urinary and fecal porphyrins, there was no significant difference between the two groups. This observation prompted the qualitative testing for the presence of blood in these urine samples by test papers (Hemastix and Occultest) and by the benzidine test (*2*). Urine sediments were examined microscopically for erythrocytes.

A definite hematuria ("4 plus") occurred in all of the experimental rats given 20 percent and, later, 40 percent ethanol in lieu of drinking water. With practically no exceptions, the hematuria persisted throughout the experimental period of 58 weeks at the end of which the rats were killed. With rare transient exceptions in older animals, the control rats, which had been given water as the sole drinking fluid, did not show hematuria. Erythocytes were observed in the urinary sediments of a number of the treated group.

With respect to the amounts of hemoglobin in the blood as determined by alkalihematin (*3*) and cyanmethemoglobin (*4*) methods, the control and experimental rats were not significantly different. The control group had averages of 16.3 ± 0.9 and 16.1 ± 1.2 g of hemoglobin per 100 ml of blood as determined by the two methods, respectively, whereas the treated group had averages of 15.9 ± 0.9 and 15.9 ± 0.8 g/100 ml, respectively. This

finding indicates that the treated group did not become anemic as a result of blood lost by way of the urinary route. However, the incidence of acute anemia in the chronic human alcoholic is not uncommon (*5*). It may be added that, in our study, three male human patients with a history of chronic alcoholism also showed hematuria. Causes of hematuria other than alcoholism were not ruled out, however. Two of these patients eventually developed other clinical complications and died. Further studies on human alcoholics are indicated.

The cause of the hematuria following administration of ethanol is not apparent at present. In a study on infectious mononucleosis in man, Custer and Smith (*6*) attributed the hematuria observed to infiltration of the kidney by lymphocytes. A possible explanation of our findings may be that ethanol induces lysis of the "old" erythrocytes by subjecting them to gross osmotic stress (*7*). Hemoglobin thus released could pass through the glomerulus and into the urine. Pertinent in this connection is a current report (*8*) that the administration of ethanol to rats leads to an increase in certain phospholipids in the liver, notably lysolecithin. Since this substance is known to lyse membranes, it seems that the hematuria herein reported could be related to an increase in the amount of lysolecithin in the liver, or perhaps in other tissues, as a consequence of ethanol ingestion.

Another possible explanation for the hematuria is that a "relative choline deficiency" develops following the prolonged administration of ethanol (*9*). Due to lack of sufficient choline, the animal may suffer renal damage and eventually pass blood into the urine. Supporting this hypothesis is the work of Valaitis *et al.* (*10*) who observed myoglobinuric nephrosis in human patients with a history of alcoholism. In a parallel study on osteomyelitis produced by *Salmonella*, hemoglobinuria was observed after the resorptive capacity of the proximal renal tubules was exceeded (*11*).

Indeed, in our study, considerable damage to the epithelial cells of the renal proximal tubule was observed microscopically in the treated group of animals. Statistically significant renal enlargement was also found in the treated rats.

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Isoprenoid Biosynthesis in a Cell-Free System from Pea Shoots

Abstract. *A cell-free system consisting of a soluble fraction and plastid fragments from pea shoots incorporates 2-^{14}C-mevalonate very actively into farnesol, squalene, geranylgeraniol, and other isoprenoids of different carbon-chain lengths. The products have different cofactor requirements, which makes it possible to channel the pathway into different products by varying the incubation mixture.*

The metabolism of 2-^{14}C-mevalonate in a cell-free system from pea shoots was studied. Such a system might be used to detect differences in the pathways of related intermediates and end products during the development of the plants. Several lines of evidence indicate a correlation between regulation of isoprenoid biosynthesis and development of plants. While seedlings are growing in the dark, cyclic triterpenoids and steroids are formed with squalene as an intermediate; but on transfer to light, the pathway is channeled into the formation of carotenoids, and the side chains of chlorophyll (phytol) and quinones instead (*1*). Inhibitors of steroid biosynthesis affect the development of plants only if they are applied at the right stage of development (*2*), a fact which indicates a different significance of the pathways at different times. Gibberellins, which are diterpenoid growth regulators with fundamental roles in plant development, accumulate preferentially during long-day conditions or at specific stages in the life cycle (*3*). Abscisin II (dormin), a sesquiterpenoid plant regulator which induces dormancy in a number of species and promotes abscission, accumulates mainly during short-day conditions (*4*).

The biosynthesis of these compounds from mevalonic acid initially proceeds along a common pathway (Fig. 1) (*1*, *5*).

The isoprenoid pathways in higher plants have been studied mainly in whole plants, excised seedlings, and tissue slices (*1*, *6*). Successful systems which incorporated substantial amounts of mevalonic acid beyond the stage of isopentenyl pyrophosphate have been derived from fruits, roots, or seeds (*7*). Some studies of mevalonic acid incorporation in cell-free systems from shoots have recently appeared (*8*).

In my work, peas (*Pisum sativum* L, "Grosse Schnabel") were grown for 10 days at 25°C under long-day conditions. The shoot tips from 1150 plants were cut off just under the third, still very small, leaf and ground with sand in 60 ml of a medium consisting of 0.45*M* sucrose, 0.1*M* tris-HCl buffer (*p*H 8.0), 0.01*M* KCl, 0.005*M* $MgCl_2$, 0.005*M* 2-mercaptoethanol, and 0.001*M* ethylenediamintetraacetic acid (EDTA). The extract was filtered and centrifuged successively at 500*g*, 1500*g*, 35,000*g*, and twice at 200,000*g*. The 35,000*g* sediment, which consisted of mitochondria and large amounts of chloroplast fragments, was washed twice in the homogenization medium and then suspended in 3 ml of a solution of 5 m*M* 2-mercaptoethanol and 1 m*M* EDTA to rupture remaining structures osmotically. This fraction is referred to as the P-35 fraction. The 200,000*g* supernatant was concentrated from 50 ml to 3 ml in a series of steps with the use of dry Sephadex G-25 to take up excess water (*9*). The concentrated solution was then purified over a column of Sephadex G-25 equilibrated with a solution of 0.01*M* tris-HCl, *p*H 7.3, with KCl, mercaptoethanol, and EDTA in the same concentrations as the homogenization mixture. The yel-