ments known to be present in the sun that it appears impossible to decide definitely about its presence, although it may reasonably be expected. The three strongest accessible lines of Tc II are all accounted for in the sun—one as a blend with Co I, one as masked by Fe I, and one as possibly present and unblended. This may furnish evidence of the presence of element 43, but the most stable known isotope, $^{99}$Tc, has a half-life of less than a million years, which is a relatively short time compared with the age of the sun. This casts doubt on the evidence. One wonders whether Tc is as rare in nature as is at present supposed.

There are many more astrophysical problems than the three special topics that have been emphasized here—atomic energy levels, the ultraviolet multiplet table, and the second revision of *Rowland's Table of Solar Spectrum Wave Lengths*. Our knowledge of forbidden lines is probably far from complete. The quantitative determination of cosmical abundances of the chemical elements will continue to attract its full share of attention as one of the most important problems. The measurement of line intensities in laboratory and stellar spectra is also of prime importance to both astronomers and physicists. Our study of atomic spectra may be well begun, but who can guess how many secrets will be revealed by the atom in the next fifty years?

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# *Technical Papers*

## *In Vitro* and *In Vivo* Production of a Ceroidlike Substance from Erythrocytes and Certain Lipids[1]


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Ceroid, an orange-brown pigmented deposit which is insoluble in alcohol, xylol, and ether, sudanophilic, and acid-fast (*1*), is found in fibrous trabeculae of cirrhotic livers of rats which have been fed a diet low in choline and its precursors. Pathological accumulation of fat in hepatic parenchyma, which always precedes and accompanies this type of fibrosis, is greatest in centrolobular regions (*2*), which are also the sites of initial fibrosis (*3*) and ceroid deposition. The same lobular area is the locus for formation of pathological fatty cysts. These atrophy and become surrounded by bands of connective tissue, so that fibrotic replacement of atrophied cysts appears to be the mechanism by which the cirrhotic lesions develop (*4*). Degeneration of a cyst may frequently be initiated by a small hemorrhage into its lumen, in which erythrocytes and lipid become intimately mixed (*5*). These red cells neither become thrombosed nor disintegrate to form hemosiderin, but it is noteworthy that it is in these regions ceroid is deposited. Furthermore, the only animals in which hemosiderin deposits in the livers could be demonstrated belonged to a special group of cirrhotic rats[2] which were largely free of ceroid. These observations suggested that, under favorable conditions, some types of lipid might react with some component of red blood cells to produce ceroid in a manner that at the same time prevented the formation of hemosiderin from the altered erythrocytes. It had been noted that some granules of ceroid resembled erythrocytes in shape and size. The possibility of making ceroid from red cells and fat was therefore attempted *in vitro* and *in vivo*.

Cod liver oil was mixed in a test tube with one tenth the volume of heparinized, washed red cells of an adult rat of the Wistar strain, and incubated for 5 days at 37° C with manual agitation at frequent intervals. The centrifuged sediment was washed repeatedly with ethyl alcohol, xylol, and ether to remove all traces of cod liver oil, affixed to gelatinized microslides, stained by a variety of methods, and examined microscopically. Many erythrocytes, although deformed, exhibited the normal staining reaction to Light Green (Color Index No. 670) and failed to show any trace of sudanophilia. Groups of others, which were often crenated, clumped, and granular, no longer stained with Light Green and were strongly sudanophilic. Aggregates of red cells altered in this


[1] This work was supported by grants from the National Cancer Institute of Canada and from the National Research Council of Canada.


[2] These animals were fed a basal choline-deficient diet supplemented with 10 mg of α-tocopherol acetate per 10 g of food mixture. The diet contained 57.5% sucrose, 12% fat, 22% protein (arachin 12%, gelatin 6%, casein 3%, fibrin 1%), with a supplement of 0.5% cystine, 1% vitamin powder (*6*), a cod liver oil concentrate, and 5% salt mixture.

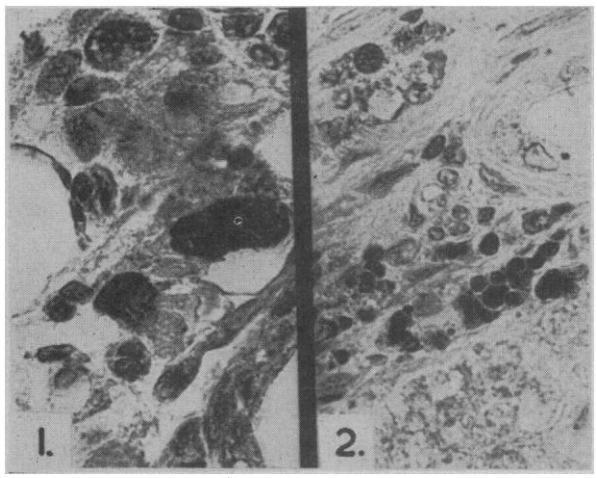


FIG. 1. Masses of ceroidlike pigment in traumatized mesenteric tissue of a rat. The sudanophilic material appears black in the photograph.

FIG. 2. Ceroid in the fibrous tissue in a section of cirrhotic liver of a choline-deficient rat. The sudanophilic pigment (black) lies in clumps which correspond to the distribution of cystic hemorrhages.

Both photomicrographs are of paraffin sections stained with Oil Red 0, hematoxylin, and Light Green. (× 650.)

manner resembled ceroid in other ways, for they were acid-fast, Prussian blue–negative, fluorescent,[3] and reacted positively to Mallory's hemofuchsin test; unstained they were orange-brown.

In a second experiment, 0.10 ml of heparin[4] was injected locally into a loose tag of fatty mesenteric tissue of each of 5 adult male rats of the Wistar strain subjected to laparotomy under ether anesthesia. A loosely tied ligature around the root of the mesenteric tag impeded venous return and systemic absorption of the anticoagulant. Fatty hematomata were produced by crushing the tissue between hemostats. The incisions were closed, penicillin was administered subcutaneously to prevent infection, and the animals were maintained on a stock diet for 10 days. Paraffin sections of the traumatized mesentery obtained at autopsy were examined by the methods used to identify ceroid. The morphology (Figs. 1, 2) and histochemistry of pigment found in the scar tissue differed in no essential from that of ceroid.

These results indicate that under certain conditions a mixture of lipids and some component of free red cells can produce ceroid or a closely related substance. Perhaps cells other than erythrocytes are capable of a similar reaction with certain lipids; this is under investigation. Preliminary experiments[5] with fat extracted from livers of choline-deficient rats have indicated that this lipid may react *in vitro* with erythrocytes to produce the same results obtained with cod liver oil. These observations may indicate means of further investigations concerning the nature not only of ceroid, but also of other lipochrome pigments.

[3] The fluorescence of ceroid in sections was first described by Popper *et al.* (7). The fluorescence of lightly colored particles of the substance produced *in vitro* above was light-brown; darker particles exhibited little fluorescence. The writer is indebted to Hans Popper, of the Hektoen Institute for Medical Research, of the Cook County Hospital, Chicago, who made the fluorescent examination for this report.

[4] Supplied in a solution of 1,000 u/ml in physiological saline by the Connaught Medical Research Laboratories, University of Toronto.

[5] These experiments and others of a similar nature have been conducted by W. G. B. Casselman, Banting and Best Department of Medical Research, University of Toronto, and will be published elsewhere.

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## An *in Vitro* Method of Screening Amebicidal Agents Using the Phillips Culture

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The testing of potential amebicidal compounds by *in vitro* methods is unfortunately beset with several difficulties. The most important is the problem of determining whether an "active" compound is truly amebicidal or whether its action is only indirect and, presumably, due to the inhibition or destruction of necessary bacterial associates. This difficulty is somewhat lessened by the use of *Endamoeba histolytica* cultures with a single bacterial symbiont instead of the usual mixed bacterial flora. However, one encounters difficulties in maintaining consistently an abundant growth level in liquid media. The use of solid media has several inherent disadvantages, the most important of which are the adsorption of the drug being tested on the surface of the solid material and the protection of amebae enmeshed in the suspended solids.

The experiment described in this report, using the bacteria-free Phillips culture (*E. histolytica* strain F 22 with *Trypanosoma cruzi*) (*1, 2*), was initiated in the belief that the use of another protozoan as a symbiont for the ameba would yield a population that would be much easier to observe and control than bacteria. Thus it was hoped that the problem of direct drug action on the amebae would be solved and disadvantages of present *in vitro* screening methods would be reduced. Various known amebicidal and chemotherapeutic agents, including antibiotics, were chosen for the test, and a comparison was made with the Stone's-Locke's egg slant (SLES) culture (*3*) currently in use in this laboratory, and the Phillips culture.

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[2] We wish to extend our appreciation to C. W. Rees, of the National Institutes of Health, for supplying us with the Phillips culture.