what. There are, however, two reasons why this is probably not the case. When hydrolysing the lipoprotein fraction and urgocyton, respectively, with N-hydrochloric acid at 100° C for 1 h, the same fluorescent substance could be isolated. The fluorescence and excitation maxima (440 and 360 mμ) of the lipoprotein fraction at *p*H 7·3 are very similar to that of the low-molecular-weight fluorescent substance at *p*H 7·6 (fluorescence maximum 430 mμ and excitation maximum 360 mμ).

Certain preliminary conclusions regarding the structure of the fluorescent substance may be drawn. Its strong fluorescence and multi-banded ultra-violet absorption spectrum indicate some unsaturated cyclic structure, an aspect which is also reflected in its low hydrogen content. The presence of one nitrogen molecule per molecular equivalence excluded the presence of purine and pyrimidine ring systems.

The optical activity of the substance makes it more interesting, since it seems unlikely that carbohydrates and amino-acids are present. The presence in it of sulphur is also worth noticing.

What is the biological significance of the bluish fluorescent substance of low molecular weight? Is it required for the proper function of the high-density lipoprotein or is it merely transported by lipoproteins and liberated in the body as such (or attached to peptides) when and where its presence is called for? Furthermore, is it attached to other proteins? What is the relationship between the lipoproteins and the fluorescent polypeptide fraction (urgocyton) isolated from liver?

The biological properties as well as the chemical structure of this substance are under investigation.

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X-Ray Crystal Data on Fluorescent Substance from Bovine Liver

A PRELIMINARY X-ray crystallographic investigation has been carried out on the fluorescent substance of unknown structure described in the preceding communication. Weissenberg diagrams were taken about the *a* and *c* axes. The crystals are orthorhombic, elongated in the *c* direction, with $a = 12{\cdot}60$ Å, $b = 17{\cdot}64$ Å, $c = 8{\cdot}51$ Å (all $\pm 0{\cdot}5$ per cent). The space group is $P\,2_12_12_1$, as reflexions $h00$, $0k0$ and $00l$ are absent for odd values of h, k and l. No other systematic absences occur. By flotation in mixtures of benzene and carbon tetrachloride the density was found to be 1·54 g cm^{-3}. This gives a weight of 439 for the asymmetric unit in the crystals. Two equivalents of weight 211 and one water molecule correspond to a weight of 440, in good agreement with the experimental value. The existence of half a molecule of water per equivalent weight was verified in drying experiments.

The space group indicates that the molecules might be optically active. They were tested directly for this, and strong activity in aqueous solution was found.

S. FURBERG

Institute of Chemistry,
University of Oslo,
Norway.

EFFECTS OF PHYTOHAEMAGGLUTININ *IN VITRO* ON CELLS OTHER THAN LYMPHOCYTES

By DR. HARRY L. IOACHIM*

Department of Pathology, College of Physicians and Surgeons of Columbia University and Francis Delafield Hospital, New York

IN 1960, Nowell demonstrated that phytohaemagglutinin (PHA), an extract of *Phaseolus vulgaris*, known for its haemagglutinating activity[1], possesses the remarkable property of being able to initiate mitoses in normal human lymphocytes *in vitro*[2]. Since this observation was made, a great number of reports from different laboratories have analysed the various facets of this phenomenon. It was found that PHA constantly produces leucoagglutination and that mitoses occur only within the clumps of white blood cells[3]. An increase in both DNA[4–7] and RNA[6–8] synthesis was demonstrated. In addition, the greater content of cytoplasmic ribosomes seen in electron micrographs indicated active protein formation[9,10]. The newly synthesized protein was identified as a γ-globulin[11]. Similarly, after PHA stimulation, the cells had increased pyroninophilia[12], acid phosphatase activity[13] and affinity for periodic acid–Schiff stains (PAS)[14].

The final result of these cellular alterations is the appearance of a different cell, larger in size, more basophilic, nucleolated and capable of mitosis having the features of a lymphoblast[10,14]. This unusual cellular transformation was interpreted in general as a response to non-specific stimulation produced by PHA[11] and as positive proof for the potentiality of lymphocytes to transform, rejuvenate and divide[15].

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No data are available as to what effect, if any, PHA may have on other types of cells in culture.

To investigate the possibility of an effect, PHA, of both types *M* and *P*, with a control of simple diluent, were added to matched subcultures of different cell types and the morphological alterations induced were studied. The types of cultured cells used are shown in Table 1. All were serial, long-term cultures, grown in Petri dishes with a constant flow of 5 per cent carbon dioxide in air, and regularly subcultured. 'Bacto-phytohaemagglutinin *M*' (code 0528, Difco Laboratory, Detroit, Michigan) was used in a concentration of 0·025 ml. per ml. medium. 'Bacto-phytohaemagglutinin *P*' (code 3110, Difco), 0·006 ml. per ml. medium, and 'Bacto-haemagglutination Buffer' (Difco), the reconstituent of lyophilized PHA, 0·025 ml. per ml. medium, were also used. PHA was added to the cultures at different times and observations were made after periods of 24–96 h, with the phase contrast microscope and on preparations fixed and stained with haematoxylin and eosin, Giemsa and PAS.

When PHA was added to cells in suspension immediately after seeding the plates, an apparent and constant agglutination occurred in all types of cultures. PHA-*P* had a more intense and rapid effect than PHA-*M*, inducing formation of larger and denser clumps (Figs. 4, 6). In contrast to control cultures where cells sedimented and attached to the glass in the following hours, in the