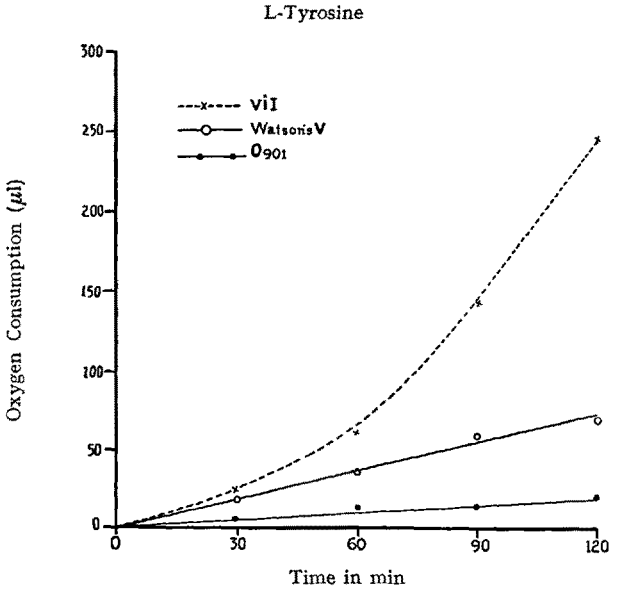

Fig. 2.—Metabolism of L-tyrosine by different antigenic strains of *S. typhosa*.

Further studies employing various substrates are in progress with a view to find a possible correlation between the enzyme make-up and the antigenic structure of strains of *S. typhosa* differently characterised.

Our thanks are due to Dr. B. MUKERJI, Director, Central Drug Research Institute, Lucknow, for his kind permission to carry on the studies reported here, in the laboratories of the Institute.

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Caius Research Laboratory, St. Xavier's College, Bombay, and Central Drug Research Institute, Lucknow, India, July 9, 1953.

Zusammenfassung

Der Metabolismus von Glutaminsäure und Tyrosin verschiedener antigener Stämme von *Salmonella typhosa* wurde studiert. Der Stamm ViI gab den höchsten Sauerstoffwert gefolgt von WATSONS V, O-901 und H-901. Es wird angenommen, dass Vi für diese Unterschiede verantwortlich gemacht werden könnte.

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An Ultraviolet Microspectrophotometric Study of the Purkinje Cells of the Adult Albino Rat[1]

It has been shown by HYDÉN[2] and BRATTGÅRD and HYDÉN[3] that the chemical composition of the PURKINJE cells of the adult animal varies quantitatively with respect to nucleic acids and proteins, and it has been stated that such variation probably corresponds to differences in the functional conditions of these cells.

It remains to be established, however, how the cytochemical features of PURKINJE cells are related to the different stages of activity. With a view to a closer approach to this problem, a preliminary investigation of the PURKINJE cells of the adult albino rat has been performed, in order to evaluate the variability in the nucleic acids and protein content of these cells under physiological conditions. To such a purpose the methods for quantitative determinations, based upon the specific absorption in ultraviolet, which have been developed by CASPERSSON[1], have been employed: in particular the intense absorption of nucleic acids at 2650 Å and the main absorption band of the average protein substances at 2800 Å have been used. The present report describes the results obtained by the photoelectric microabsorption technique. A detailed discussion of the results will be published elsewhere.

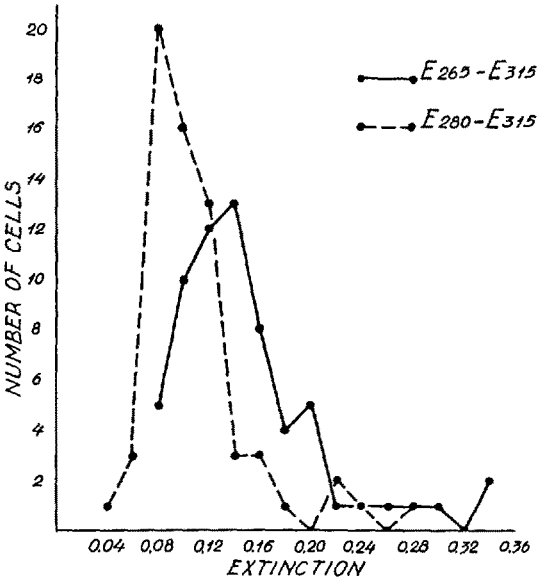

Fig. 1.—Frequency distribution of ultraviolet extinction values at 2650 Å and 2800 Å of PURKINJE cells, "corrected" by the subtraction of the extinction at 3150 Å: mean cytoplasmic values.

Material and methods. The anterior lobe of vermis cerebelli of an adult albino rat was treated in accordance with the freezing-drying method, embedded in paraffin and sliced in sections 5µ thick. The absorption measurements were carried out on the sections immersed in glycerine by the universal ultramicrospectrograph, which has recently been developed by CASPERSSON[2]. The diameter of the area in the object which was projected into the photomultiplier tube was about 0·77 µ. In order to obtain a mean extinction value for the cytoplasm of each cell it was considered sufficient to carry out the measurement by the scanning device, along a single cytoplasmic track. The cells selected for measurement were those which contained in the section the major part of the nucleus and which showed no signs of shrinkage. The transmission curves obtained with the microspectrograph were transformed into extinction curves by means of an automatic extinction calculator, while an integrator arrangement registered in the same time the surface under the extinction curve, that is the total extinction

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[3] S. O. BRATTGÅRD and H. HYDÉN, Acta Radiolog. Suppl. *94* (1952).

[1] T. CASPERSSON, Skand. Arch. Physiol. *73*, Suppl. 8 (1936); J. Roy. Micr. Soc. *60*, 8 (1940); *Cell growth and cell function*. W. W. Norton Co., New York, 1950.
[2] T. CASPERSSON, Exp. Cell. Res. *1*, 595 (1950). – T. CASPERSSON, F. JACOBSSON, and G. LOMAKKA, Exp. Cell. Res. *2*, 301 (1951).

Ultraviolet extinction values of the cytoplasm of PURKINJE cells before and after digestion by ribonuclease, and before and after extraction with water

Cytoplasm Cell No.		$E_{265}-E_{315}$			$E_{280}-E_{315}$			E_{315}		
		Before	After	Variation %	Before	After	Variation %	Before	After	Variation %
Ribonuclease digestion	55	0·252	0·063	−75	0·164	0·05	−69·5	0·070	0·075	+ 7·14
	56	0·172	0·03	−82·6	0·109	0·017	−84·4	0·069	0·074	+ 7·25
	57	0·214	0·066	−69·15	0·157	0·048	−69·4	0·077	0·076	− 1·3
	58	0·188	0·048	−74·47	0·143	0·034	−76·2	0·074	0·137	+85·1
	59	0·182	0·015	−91·76	0·128	0·025	−80·5	0·059	0·089	+50·8
	60	0·137	0·007	−94·9	0·089	0·017	−80·9	0·067	0·086	+28·4
Water extraction	61	0·137	0·086	−37·2	0·124	0·070	−43·6	0·029	0·050	+72·4
	62	0·147	0·138	− 6·1	0·116	0·121	+ 4·3	0·096	0·114	+18·7
	63	0·164	0·067	−59·1	0·144	0·064	−55·6	0·062	0·083	+33·9
	64	0·090	0·067	−25·5	0·067	0·060	−10·4	0·050	0·068	+36
	65	0·076	0·061	−19·7	0·074	0·071	− 4·1	0·057	0·050	−12·3

along the cytoplasmic track: from this and the length of the track the average extinction coefficient was calculated.

65 PURKINJE cells were measured with the ultramicrospectrograph, at three wavelengths, namely at 2650 Å, very near the nucleotide absorption maximum, at 2800 Å, i.e. at the protein absorption maximum and at 3150 Å, where the specific chemical absorption is practically nil and only a non-specific light loss, due to scattering and reflection in the preparation, is thought to occur. Moreover, for a few cells a complete absorption spectrum was determined.

Some cells were measured along the same track before and after the digestion with WORTHINGTON crystalline ribonuclease (0·2 mg/1 cm³ distilled water) for 2 h at 37° C. In order to free the enzyme from any possible proteolytic activity, the ribonuclease was boiled in saturated ammonium sulphate, according to the procedure proposed by MC DONALD[1]. Some other cells were measured before and after extraction with distilled water at 37°C for 2 h.

Results and discussion. The frequency distribution of ultraviolet extinction values at 2650 and 2800 Å for the cytoplasm of the measured cells is presented in Figure 1: the values have been "corrected" by the subtraction of the extinction at 3150 Å (on the arbitrary assumption that the observed E_{315} represents a greater part of non-specific light losses at all wavelengths[2]). One single cell, not included in the graph, has shown very high extinction values, namely $E_{265}-E_{315} = 0{\cdot}478$ and $E_{280}-E_{315} = 0{\cdot}377$. It appears from the graph that the modal extinction value at 2650 Å is in the neighborhood of 0·14, whereas that at 2800 Å approximates 0·1. A marked spread of extinction values is clearly evident: it is probably due mainly to biological variability, to a lesser extent to the effects of the preparatory treatment. The spread of individual observations appears also from the high sigma values (respectively 0·07 for the data of $E_{265}-E_{315}$ and 0·054 for those of $E_{280}-E_{315}$). The distribution curve shows a positive skewness with its mode to the left of the mean.

[1] M. R. MC DONALD, J. Gen. Physiol. *32*, 39 (1948).

[2] It is actually difficult to estimate in the individual cases the proportions of the light-losses due to reflection (independent of wavelength) and to scattering in the preparation: the latter, on the other hand, according to the results of the ribonuclease tests (see later), does not seem to vary, in this material, in inverse proportion with the 4th power of the wavelength (RAYLEIGH's formula), but rather with a lower power of lambda, even if we assume that it contributes only for one half to the loss of light at 3150 Å.

The study of the absorption spectra has shown a distinct nucleic acid band and a protein band, even in the curves taken from very slightly absorbing cells (Fig. 2).

In the Table the ultraviolet extinction values of the cytoplasm of some PURKINJE cells are recorded which were measured before and after digestion by ribonuclease, and before and after extraction with water respectively. In view of the shrinkage caused by the action of temperature and the dehydration, it was found advisable to correct the data for $E_{265}-E_{315}$ and $E_{280}-E_{315}$, obtained after treatment with ribonuclease and water, by reducing them in the same percentage proportion in which the cell section areas appeared to be reduced: such a correction on the contrary was not made for the E_{315} values, as it was difficult to evaluate the influence of the decrease in cell volume upon the aspecific losses of light.

It appears from the table that the extinction at 3150 Å has more or less increased after digestion by ribonucle-

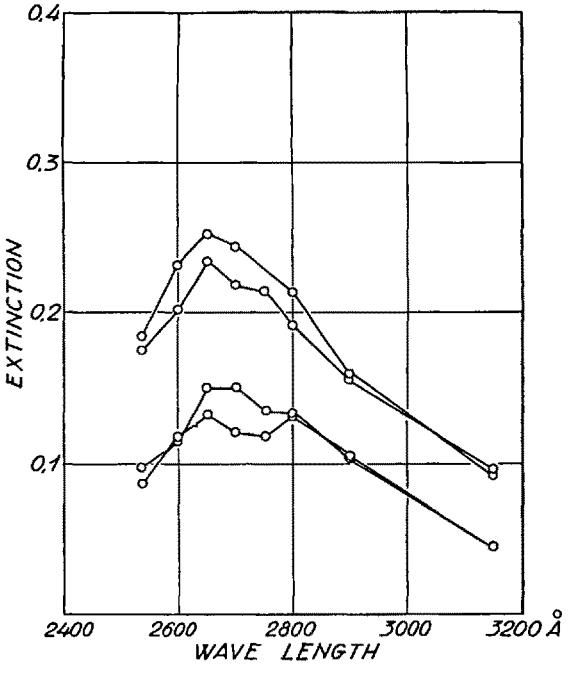

Fig. 2.—Absorption spectra from the cytoplasm of different PURKINJE cells (mean values). All curves show a nucleotide band at 2650 Å and a protein band at 2750 or 2800 Å.

ase, sometimes even in a great degree: this is probably due to the aggregation of the protein particles in larger masses as a result of the action of the temperature: the action of the enzyme on the contrary seems to be of no importance in this respect, since an increase of the extinction values ranging within the same limits has been observed also after extraction with water.

The values of E_{265}-E_{315} appear to be markedly reduced after digestion by ribonuclease; the values of E_{280}-E_{315} too are decreased, in the same or in a slightly lesser degree than the former. Also after extraction with water there is a more or less considerable reduction of the extinction values, but it is in any way much lesser than after treatment with ribonuclease. This large variability in the effects of water extraction, which does not appear to be in a close relationship with the initial extinction values, makes it impossible to establish, in the case of digestion by ribonuclease, how much is due to the action of the enzyme and how much to the water extraction. On the other hand, it should be noted that, according to BRATTGÅRD and HYDÉN[1], the digestion by ribonuclease cannot be compared with a simple water extraction, since the enzyme has a denaturing effect on the protein structures; in fact, after treatment with ribonuclease, those authors did not observe any loss in weight, determined by X-ray microradiography, other than that which could be referred to the action of the enzyme. On the basis of this assumption, the marked reduction of the extinction values at 2650 Å, after digestion by the enzyme, is due to the remotion of PNA and, in a much lesser degree, to the release of the proteins, which are attached to the PNA and which go into solution together with the splitting off of it; the reduction of the extinction at 2800 Å, on the other hand, must be referred to the same factors, except that the release of the proteins bound to the PNA has, in such regard, a greater influence at this wavelength than at 2650 Å. A direct action of the ribonuclease on the proteins, on the contrary, should not be admitted, since the enzyme used in the present work had to be considered free from proteolytic activity.

I wish to express my great indebtedness to Prof. T. CASPERSSON, Director of the Institute for Cell Research and Genetics, for his generous hospitality and valuable advice and interest. My sincere thanks are also due to Dr. G. MOBERGER for kind help and to Ing. J. KUDYNOWSKI for technical assistance.

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Résumé

L'auteur a fait une étude microspectrophotométrique des cellules de PURKINJE du rat blanc adulte. La courbe de distribution des fréquences montre une remarquable dispersion des valeurs d'extinction cytoplasmiques à 2650 Å et 2800 Å, avec un groupement des fréquences plus hautes vers les valeurs basses. L'étude des spectres d'absorption a démontré un maximum d'absorption des nucléotides et un maximum protéique, même dans le cytoplasme des cellules moins absorbantes.

La digestion des coupes par une ribonucléase dépourvue d'activité protéolytique a provoqué une diminution considérable des valeurs d'extinction cytoplasmique à 2650 Å et 2800 Å; aux mêmes longueurs d'onde l'extraction par l'eau distillée a déterminé une réduction d'extinction très variable, mais en tout cas bien inférieure à celle provoquée par l'enzyme. Après la digestion par la ribonucléase, ainsi qu'après l'extraction par l'eau, on a observé une augmentation plus ou moins remarquable des pertes non spécifiques de lumière dues à la diffraction et à la réflexion dans la préparation.

[1] S. O. BRATTGÅRD and H. HYDÉN, Acta Radiol. Suppl. *94* (1952).

[2] *Present address:* Institute of Histology and General Embryology, University of Padua.

A Model for the Cortical Reaction of Fertilization in the Sea-Urchin Egg

The disappearence of the cortical birefringence of the sea-urchin egg as a result of fertilization[1] has been tentatively interpreted as a disarrangement of the submicroscopic structure due to the splitting of the lipoprotein complex of the cortical layer through the action of some agent introduced by the sperm[2].

To check this hypothesis, a model experiment has been devised. As a model for the cortical layer of the unfertilized sea-urchin egg, the lipoprotein of the hen's egg–lipovitellin–has been prepared according to CHARGAFF[3]. Dyalisis of the lipovitellin against sea-water results in a whitish emulsion. To this a fresh suspension of sperm of *Arbacia lixula* was added and at various intervals of time (from 2 to 15 min) free phospholipids were extracted with cold ether. The extract was evaporated, dried with anhydrous Na-sulphate and redissolved in ether and chloroform. After evaporation, lipid-P was determined. Zero time tubes were extracted with cold ether immediately upon addition of sperm to the lipovitellin. A certain amount of ether-extractable phospholipids was present in our preparations of lipovitellin. However, no further spontaneous release of phospholipids occurred on standing at room temperature for the time of the experiment. Therefore, together with each experiment a control was run in which sea-water was added to the lipovitellin instead of sperm.

μg of phospholipid-P released from lipovitellin under the influence of sperm of *Arbacia lixula*

Control (lipovitellin)	Time after mixing, in minutes				
	0	2	5	10	15
10·5	22·2	27·4			
12·8	29·0		35·0		
22·4	32·4			45·4	
15·2	32·4				44·4
9·8	12·2				15·1

The results of the experiments are summarized in the Table. They show that under the influence of sperm, phospholipids are released from the lipovitellin. This model–although certainly a very rough one–at least indicates that sperm are endowed with mechanisms which enable them to break-up lipoprotein bonds, thus releasing the phospholipids. That may be considered to support our interpretation of the cortical reaction of fertilization in the sea-urchin egg.

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