lymph glands of 120 h old larvae (referring to the time of egg laying) had been investigated under the same culture conditions. According to BARIGOZZI, CASTIGLIONI, and DI PASQUALE[11], the lymph glands of non-etherized larvae were dissected in physiological solution, embedded in agar, fixed in Carnoy's fluid and mounted in euparal. Of every stock more than thirty lymph glands had been investigated.

The lymph glands of Berlin wild larvae proved to be intact; less intact seem to be the lymph glands of e^{11} and *yw*, while the least intact are those of tu^{g} and se e^{11} tu^{49h}. Apparently they release groups of cells into the hemolymph. The dissolution of the lymph glands increases as seen in the Table.

The Berlin wild, e^{11}, and *yw* stocks are genetically tumorless stocks as seen by dissection of 5000 adults[5] and recent investigating of more than 200 third-instar-larvae of every stock. The incidence of hereditary tumors is about 70% in tu^{g} and 60% in se e^{11} tu^{49h}. The morphological structures of some characteristic types of the investigated lymph glands are shown in the Figure.

The results show a difference in the state of the lymph glands between the Berlin wild and *yw* stocks. Since all the tested tumor stocks have loose lymph glands, the loose structure of the lymph glands may be one of the reasons for the reported host specificity, which explains why one can induce tumors more successfully by injecting cellfree extracts in *yw* than in the Berlin wild stock which has intact glands. Certainly it may be not the only reason for this effect. Other experiments are now under way to clarify the host specificity reported above[13].

Zusammenfassung. Die larvalen Lymphdrüsen von 3 tumorfreien und 2 erblich tumorösen Stämmen von *Drosophila melanogaster* wurden untersucht, um Zusammenhänge zwischen dem Auftreten erblicher und durch Injektion zellfreier Extrakte induzierter melanotischer Bildungen zu prüfen. Es wurde gefunden, dass die Lymphdrüsen der getesteten erblich tumorösen Stämme stärkere Auflösungserscheinungen zeigen als die der tumorfreien Teststämme. Die im Vergleich zu Berlin wild lockerere Struktur der Lymphdrüsen von *yw* wird als mitverantwortlich für die bei den Versuchen zur Induktion melanotischer Bildungen in *yw* (positiv) und Berlin wild (negativ) beobachtete Wirtsspezifität angesehen.

G. RÖHRBORN

Institut für Genetik der Freien Universität Berlin, Berlin-Dahlem (Germany), June 14, 1961.

[13] The author wants to express his gratitude to the 'Consiglio Nazionale delle Ricerche, Roma', for a grant, which permitted him to carry out this investigation in the Department of Genetics of the University of Milan, to Prof. C. BARIGOZZI for his kind hospitality, and to Prof. M. C. CASTIGLIONI for her technical advice.

The *in vitro* Effect of Various Enzymes upon the Mouse Ascites Tumor of Ehrlich[1]

In previous experiments it was noted that the malignancy of ascites tumor cells decreased appreciably after incubation with α-amylase[2]. Additional studies concerning the effect of other enzymes on the malignancy of the ascites tumor cells were therefore undertaken.

Ascites tumor cells (Ehrlich) were harvested from tumor bearing, white Swiss mice (20–22 g) 7 days after inoculation. These cells were centrifuged at 4000 rpm for 10 min, then washed twice with normal saline equal in volume to the packed cells. The cells were then suspended in twice their volume of saline, the enzyme under investigation was added, and the mixture was incubated at 37° in a water bath equipped with a shaker (90 motions/min). In each experiment a control mixture was incubated without enzyme.

After 9 h incubation, aliquots from each mixture were used for cell counts in a hemocytometer, for eosin resistance tests according to the method of SCHRECK[3], for the preparation of cell smears, and for the injection of 10 normal mice in order to ascertain their ability to produce tumors. According to the cell counts per unit volume of each mixture, 8.5×10^6 cells were used for each injection. This number was considerably in excess of that needed for the passage of tumor cells (1×10^6) cells. The mice were inspected every 3 days for tumor development and were finally autopsied.

The pH of the tumor cell suspension varied from 7.2 to 7.4, and did not change during incubation more than 0.2 units. Although this is not the optimal pH for some of the enzymes which were investigated, it was decided not to add buffers and alter the pH, in order to avoid introduction of another variable. Moreover, the amount of enzyme used was as a rule quite large. All the enzyme preparations used were purchased from Worthington Biochemical Corporation (New Jersey).

Cell counts, carried out on the cell suspensions at the beginning and end of the incubation, showed no appreciable change in number of cells.

In the following tabulation, the results of various enzymes on the malignancy of the mouse ascites tumor cells are summarized:

(1) Enzymes which did not show any effect upon the malignancy of the tumor cells—i.e., both experimental and control groups had similar survival curves, with 100% accumulated deaths after 16–22 days (Table I).

(2) Enzymes which caused a loss of virulence of the mouse ascites tumor cells—i.e., no evidence of tumor 40 days after inoculation of the experimental group but 100% mortality in control animals (Table II).

Cytological studies were carried out on the various tumor cell preparations.

(a) Eosin-resistance of enzyme-treated cells is shown in Table III.

(b) Duplicate cell smears from mixtures treated by the enzymes in Group 2 were stained by the Papanicolaou method, and with haematoxylin and eosin. They were

Tab. I

Elastase	10[a]	Cathepsin	50	Lysosyme	25
Catalase	50	Carboxypeptidase	10	Collagenase	20
Peroxidase	10	Lipoxydase	25	β-Amylase	30
Lipase	50	Hyaluronidase	25	Trypsin	25

[a] mg/3 cm³ of saline cell suspension, containing about 34×10^7 cells, 3 experiments.

[1] This work was supported in part by a grant from the American Cancer Society (Mass. Division) and in part by grants (C-3538 and CY-4018) from the U.S. Public Health Service, National Cancer Institute.

[2] F. STECKERL, A. OFODILE, R. R. CAMPBELL, and G. H. FRIEDELL, Nature, in press (1961).

[3] R. SCHREK, Amer. J. Cancer *28*, 389 (1936).

examined without knowledge as to treatment previously given the incubation mixture. Depending on the appearance of their nuclei, cells were considered to be either viable or non-viable and were tabulated in these two categories. About 25% of the cells of the control preparations were considered to have remained viable at 9 h. After a similar period of incubation with ribonuclease, 50% appeared viable, 80% when chymotrypsin was used, 75% when glucuronidase was used, and approximately 10% when cellulase and arginase were used. In the above experiments, despite the fact that ribonuclease, chymotrypsin and β-glucuronidase markedly altered the malignancy of tumor cells *in vivo*, cytologically more cells appeared viable after *in vitro* incubation than in the control preparations.

Apparently only during starvation and ageing do ascites tumor cells become permeable to the enzyme-proteins. Therefore, the failure of enzymes in Group 1 to affect the tumor virulence need not indicate that the structures, which may be hydrolysed by these enzymes, are non-essential parts of the tumor cells or even absent. The ineffectiveness may be due to permeability barriers on the cell surface, lack of access to the structures in intracellular compartments, or the presence of inhibitors. The interesting difference between the effect of chymotrypsin and trypsin can probably be thus explained, since from a purely enzymatic point of view, these two enzymes do not seem to differ as much as their specificities as was originally thought[4].

Tab. II

Chymotrypsin	25[a]	Arginase	50
Ribonuclease	50	Cellulase	50
β-Glucuronidase	50		

[a] mg/3 cm³ of tumor cell suspension, containing about 34 × 10⁷, 6 experiments.

Tab. III

Group	h of Incubation	% Eosin-resistant cells
Control	0	85–95
Control	7	80
Enzymes in Group 1	7	70–75
Enzymes in Group 2	7	
Chymotrypsin		50
Ribonuclease		5
Arginase		75
Cellulase		10
Glucuronidase		60

The arginase, β-glucuronidase and cellulase were not pure preparations, and consequently, their effect might possibly have been due to the enzymatic activity of contaminating proteins. In experiments not reported here, we found no evidence of the presence of cellulose in these tumor cells. However, the findings with chymotrypsin and ribonuclease, which are pure preparations, like the results from our previous studies with α-amylase, indicate that a damage to a variety of cellular structures may be quite deleterious to the growth potential of these tumor cells.

Cytological studies, as indicated above, show that inactivation of specific cellular structures to a degree sufficient to prevent the growth of injected tumor cells may not be adequately reflected either by study of fixed and stained preparations or of non-fixed eosin stained preparations. When cell damage was apparent, one could not differentiate specific injury from non-specific.

Moreover, there was a marked discrepancy between the number of presumably viable cells on the basis of the viability criteria of eosin resistance and the number of presumably viable cells on the basis of the microscopical appearance of the cell nuclei. We apparently lack good morphological criteria by which to decide whether or not a cell is capable of multiplication. It has been observed that cells which showed profound changes in their microscopical appearance, may recover completely[5]. On the other hand, cells damaged by a certain amount of radiation may still pass through one or more divisions, although they have lost the power of indefinite proliferation[6]. Thus, it would be hazardous to explain the above findings concerning cell damage through enzyme action by the ratio of presumably viable to presumably non-viable cells. The morphological criteria of viability are apparently not fine enough to ascertain the character as well as the reversibility of cell damage.

Zusammenfassung. Die Virulenz von Ehrlich-Ascitestumorzellen wurde durch Inkubation mit fünf Enzymen *in vitro* stark herabgesetzt. Zwölf andere Enzyme hatten keine Wirkung. Die morphologischen Kriterien für Zellschädigung werden erörtert.

F. STECKERL, A. OFODILE,
R. R. CAMPBELL, M. L. TURNER,
and G. H. FRIEDELL

Department of Medicine and Pathology, Boston, University School of Medicine, Boston (Massachusetts), August 21, 1961.

[4] T. INAGAMI and J. M. STURTEVANT, J. biol. Chem. *235*, 1019 (1960).
[5] H. EAGLE, J. exp. Med. *102*, 37 (1955).
[6] L. H. GRAY, Radiation Res. Suppl. I (1959).

Nachweis von Corticosteroiden in menschlicher Placenta und Isolierung von 16α-Hydroxytestosteron[1]

Während der Gravidität kommt es zu einem erheblichen Anstieg des Blut- und Urinspiegels der Glucocorticoide[2–7] wie der Mineralcorticoide, insbesondere des Aldosterons[7–14]. Als Ursache scheint nicht so sehr eine erhöhte adrenale Sekretion als vielmehr ein verminderter Katabolismus des mütterlichen Organismus verantwortlich zu sein[15–17], wobei offen bleibt, ob eine Veränderung in Konjugation oder Clearance ebenfalls eine Rolle spielt.

Wie weit die Placenta selbst als Bildungsort für Corticosteroide in Frage kommt, liess sich aus den wohl zum Teil durch *species*-Unterschiede bedingten Befunden für [18–28] und gegen [15,17,29–31] eine solche Möglichkeit nicht erkennen.

In diesem Zusammenhang lag uns zunächst daran, in Nachprüfung verschiedener Literaturangaben[22,25,32–38] die Corticosteroide, insbesondere Aldosteron, in Placenten nachzuweisen.

Methoden. Der aus je 1 kg Gewebe erhaltene Acetonextrakt wurde durch Verteilung zwischen 70prozentigem Äthanol und Hexan entfettet und präparativ papierchromatographiert (Formamid/$CHCl_3$); nach Elution der