lyophilisée, l'activité se maintient pendant au moins 2 mois. Le résultat de l'analyse élémentaire et du dosage des héxoses et héxosamines est résumé dans le Tableau II. A l'encontre de l'hormone placentaire protéique (gonadotrophine choriale) isolée récemment par BOURILLON et GOT[11], le P.P.F. se distingue par un pourcentage en azote nettement plus faible (6,52% à la place de 10,5% trouvé par GOT) et un taux en substances glucidiques plus élevé (41% à la place de 19,7%, GOT). L'analyse qualitative des acides aminés par chromatographie après hydrolyse en tube scellé révèle la présence d'au moins 7–10 acides aminés.

Tab. II. Caractères analytiques du P.P.F. (facteur inhibiteur et hyperémie)

Activité biologique (unités rate/mg)	25000–50000
N (%)	6,52
C (%)	46,68
H (%)	7,3
Hexoses (%)	26,0
Hexosamines (%)	15,0
Acides aminés, approximativement	7–10

Nous en concluons que le P.P.F. (facteur d'inhibition utérine et d'hyperémie ovarique) est, dans sa forme actuelle, un complex formé d'acides aminés et de sucres avec un poids moléculaire relativement peu élevé.

Summary. Description of a process for extraction and isolation of a placental hormone with the following biological and chemical properties: (1) Inhibition of the spontaneous contractions of the isolated rat-uterus with 0.05 γ/ml. (2) An ovarian hyperaemia reaction is produced by intraperitoneal injection of 0.01–0.025 γ to rats weighing between 35 and 45 g. (3) The biologically active product is of comparatively low molecular weight. Analytical research suggests as principal constituents amino-acids and sugars.

O. STAMM

Clinique universitaire de Gynécologie et d'Obstétrique, Genève, Centre de transfusion et Laboratoire central de l'Hôpital cantonal, Genève (Suisse), le 15 mai 1961.

[11] R. BOURILLON, R. GOT et R. MARCY, Bull. Soc. Chim. biol. *41*, 267 (1959).

Drosophila Tumors and the Structure of Larval Lymph Glands

Hereditary *Drosophila* tumors are usually melanotic formations which arise in the larvae through an aggregation and subsequent melanization of lymph cells. During the larval or pupal stage, the cellular character of these formations disappears, and the adults show only easily detectable pigment masses. Phenocopies of these tumors can be induced by several substances[1,2]. As shown in former experiments[3–5], they also can be induced very frequentily (about 70%) by injecting acellular larval extracts of tumorous (*tu^g*, se *e^{11} tu^{49h}*) and tumorless (*e^{11}*) stocks of *D. melanogaster* into larvae of the tumorless *yw* stock.

The same extracts failed to induce such phenocopies in Berlin wild stock; this means that a host specificity exists for the induction of melanotic formations in *Drosophila*. In *yw* even an injection of physiological solution succeeded in inducing a small but significant number (18.4%) of melanotic tumors, demonstrating how easy it is to induce such formations in this stock. The production of these formations is probably caused by a defence reaction of the insect, as was also shown by WALKER[6] in investigations with the parasite *Pseudocoila bochei*.

The problem is to show what structure or what kind of mechanism is responsible for the different reactions of *yw* and Berlin wild to the injection of acellular extracts. The present paper deals with the first part of a series of different investigations aimed at clarifying this problem.

The structure of the lymph gland of *Drosophila* larvae has been described by several authors[7,8]. This gland is situated near the dorsal vessel and consists of pairs of lobes on both sides of this vessel. As seen in Figure a, the first two pairs of lobes are usually bigger than the other pairs. Because the lymph gland produces the lymph cells, it is also often called the blood-forming organ. As reported by some investigators[9–12], the structure of the lymph gland is generally more intact in tumorless than in tumorous stocks, even though also tumorless stocks can have very loose lymph glands. On the other hand, the authors found no tumorous stock with completely intact lymph glands. Therefore, a loose character of the lymph gland seems to be necessary for producing tumors in *Drosophila*, although it is undoubtedly not the only prerequisite for their formation.

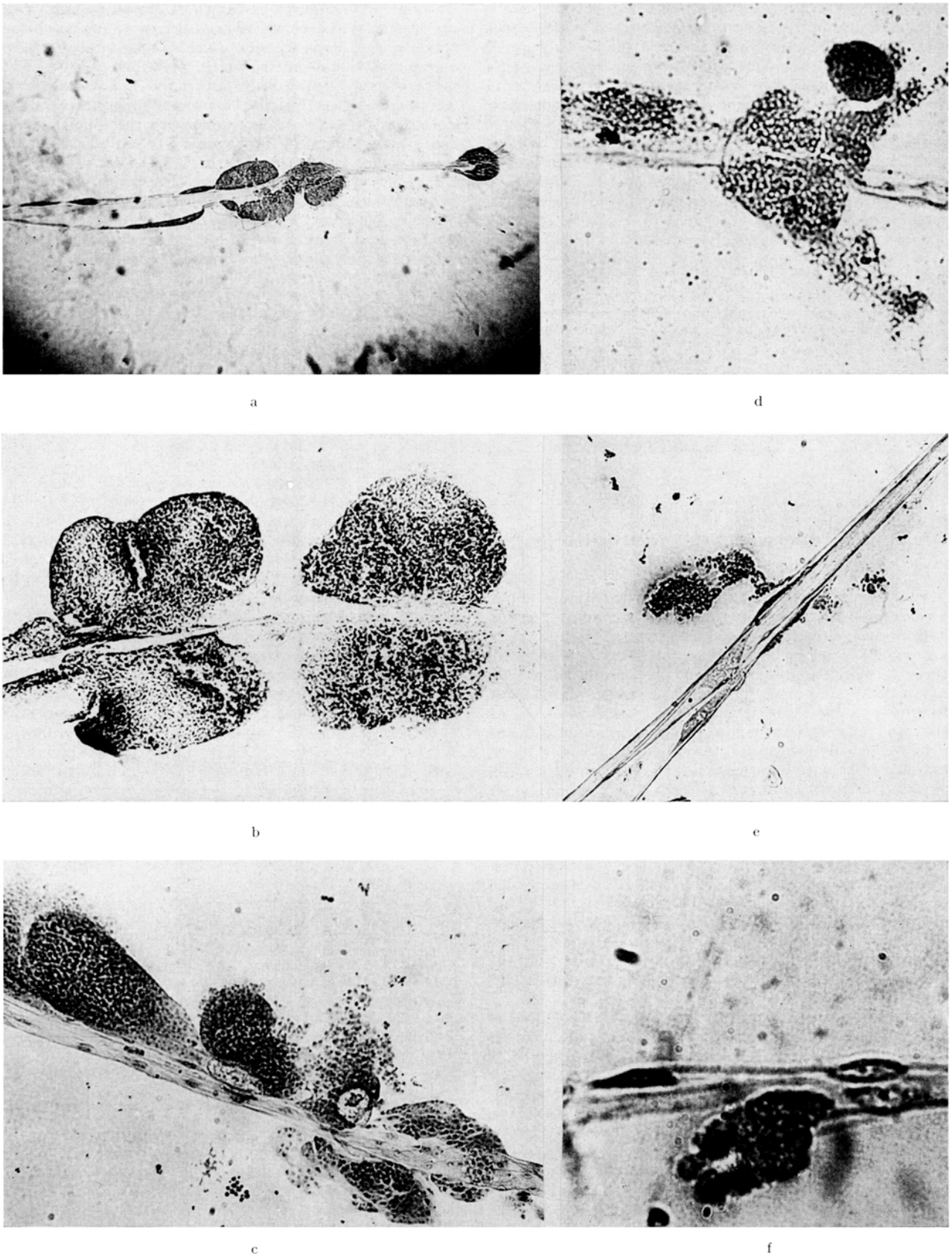
In the present investigation, I examined lymph glands of the following tumorless and tumorous stocks which I used in the induction experiments: *yw*, Berlin wild (hosts), *tu^g*, se *e^{11} tu^{49h}*, *e^{11}* (donors). To prevent the age of the larvae and environmental factors from influencing the structure of the lymph glands in a different manner, the

Lymph gland structure and tumor penetrance.

Stocks[a]	% tumor incidence
Berlin wild	0
e^{11}	0
yw	0
tu^g	± 70
se *e^{11} tu^{49h}*	± 60

[a] The column is arranged so as to classify the lymph gland structure from the compact (top) to the very loose (bottom). Differences are slight between *e^{11}* and *yw*, and between *tu^g* and se *e^{11} tu^{49h}*; greater between Berlin wild and *e^{11}*, and between *yw* and *tu^g*.

[1] M. C. CASTIGLIONI and S. BEATI, Exper. *10*, 501 (1954).
[2] M. C. CASTIGLIONI, Atti II Riunione A.G.I., La Ricerca Scientifica, Suppl. *26*, 125 (1957).
[3] G. RÖHRBORN, Diplomarbeit, Humboldt-Universität, Berlin (1955).
[4] G. RÖHRBORN, Dros. Inf. Serv. *30*, 148 (1956).
[5] G. RÖHRBORN, Arch. Entw. mech. Org. *150*, 115 (1957).
[6] I. WALKER, Rev. Suisse Zool. *66*, 569 (1959).
[7] M. B. STARK and A. K. MARSHALL, J. Amer. Inst. Homeopathy *23*, 1204 (1930).
[8] H. H. EL SHATOURY, Arch. Entw. mech. Org. *147*, 489 (1955).
[9] C. BARIGOZZI, J. cell. comp. Physiol. *52*, 371 (1958).
[10] C. BARIGOZZI, M. C. CASTIGLIONI, and A. DI PASQUALE, Exper. *14*, 443 (1958).
[11] C. BARIGOZZI, M. C. CASTIGLIONI, and A. DI PASQUALE, Heredity *14*, 151 (1960).
[12] C. BARIGOZZI, M. C. CASTIGLIONI, and A. DI PASQUALE, ACTA *16*, 53 (1960).



(a) Ring gland (r) and total lymph gland of Berlin wild (× 44); (b)–(f) lymph glands of Berlin wild (b), e^{11} (c), *yw* (d), *tus* (e), and se e^{11} tu^{49h} (f) (× 125).

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).