

The graft of egg albumen imbibed with saline (Gey solution or Holtfreter solution) does not induce any important change in chorioallantoic membrane, while the graft of egg albumen containing RNP<sub>H</sub> induces the appearance of many flask-shaped or elliptical cells with intensely stainable cytoplasm and some multinucleate muscular fibers with fibrillar cytoplasm (Figure 2). We may therefore conclude that RNP extracted from heart can induce the formation of muscular fibers. The graft of egg albumen with RNP<sub>L</sub> can induce the transformation of the epithelial cells adherent to the grafted albumen into characteristic tissue made up by polygonal cells piled up into parallel cords; some of these cells may be free in the mesenchymatic tissue (Figure 3). Consequently we may conclude that RNP extracted from liver induces the formation of liver cells.

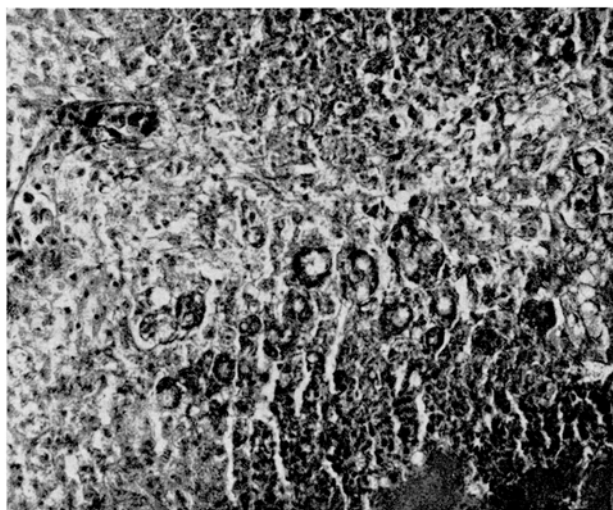


Fig. 3. Glandular structures induced by liver ribonucleoprotein. The graft is at the bottom of the picture ( $\times 274$ ).

### The Heterogeneity of Serum Glutamic-oxalacetic Transaminase of Certain Mammalian Species

Several enzymes have proved to be heterogeneous when analysed by electrophoresis or chromatography<sup>1</sup>. These molecular forms (isozymes) of an enzyme, being similar in enzymic activity, exhibit characteristic patterns of distribution in each organ of an organism. During a study on esterases and transaminases in blood plasma (serum) of pigs with hepatic lesions<sup>2</sup>, the electrophoretic pattern of certain sera from pigs with high glutamic-oxalacetic transaminase (GOT) activity was characterized by two fractions with GOT activity. Meanwhile, FLEISHER et al.<sup>3</sup> reported two fractions with GOT activity in crude extracts of heart and liver (man, dog, pig); after completion of the present work, the same authors<sup>4</sup> announced their observation of two GOT-active fractions in serum of dogs following acute injury of the liver. In addition, JUNGNER<sup>5</sup> reported briefly that, depending on the pH used on electrophoresis, GOT activity is detected in two or three fractions of human serum. The heterogeneity of serum GOT of certain other mammalian species will be briefly reported.

We have studied also the action of RNP extracted from frog (*Rana esculenta*) liver and we have been able to show that this RNP is able to induce, in the chorioallantois of chick embryo, the same kind of tissue induced by the RNP extracted from the chicken liver.

After having imbibed a piece of albumen with chicken RNP, we heated it in order to denature the RNP, putting the whole thing in a test tube in boiling water for 10 min before implanting it in the chorioallantois. The amount of induced tissues was enormously reduced and only few or no cells were transformed.

We may therefore conclude: some thermolabile substances which are able to induce organospecific cell differentiation, are present in our RNP preparations. Our RNP preparations are therefore organospecific: i.e. the heart RNP induces muscular structures; the liver RNP induces glandular tissue. The organospecificity is more effective than the speciespecificity: i.e. frog liver RNP can induce in the chick embryo the same tissue induced by the chicken RNP<sub>L</sub><sup>4</sup>.

**Riassunto.** Preparati di RNP di cuore o di fegato di pollo impiantati (in albume coagulato) su membrana corioallantoidea (Figura 1) inducono, in questa, formazione rispettivamente di fibre muscolari (Figura 2) e di tessuto ghiandolare (Figura 3). Il principio attivo viene distrutto con cottura a bagno maria. RNP di fegato di rana è in grado di indurre nella membrana corioallantoidea di pollo tessuto ghiandolare identico a quello indotto da RNP estratto dal fegato di pollo.

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<sup>4</sup> After we sent this paper to the Editor, we found by the same method that: RNP extracted from frog heart induces the same structures induced by RNP from chick heart; RNP extracted from chick skeletal muscles induces the formation of a tissue different from that induced by RNP from heart, because the nuclei are peripheral in the so-formed structures; RNP extracted from chick kidney induces the formation of tubular structures and of some other structures tentatively interpreted as glomeruli.

Blood samples were taken from individuals with known high transaminase activity. GOT and GPT (glutamic-pyruvic transaminase) were determined according to a method described by KARMEN et al.<sup>6</sup> as modified by ORDELL<sup>7</sup>. Transaminase activity was assayed at 340 mμ with a Beckman B spectrophotometer and expressed as  $\Delta A \times 10^3/\text{min/ml}$  serum. The activity of fractionated serum after electrophoresis was similarly expressed per ml fraction.

<sup>1</sup> Conference on multiple molecular forms of enzymes. Ann. N.Y. Acad. Sci., in press.

<sup>2</sup> K.-B. AUGUSTINSSON, C. A. GRANT, B. OLSSON, and B. THAFVELIN, Zentr. Vet.-Med. 7, 729 (1960).

<sup>3</sup> G. A. FLEISHER, C. S. POTTER, and K. G. WAKIM, Proc. Soc. exp. Biol. Med. 103, 229 (1960).

<sup>4</sup> G. A. FLEISHER and K. G. WAKIM, Proc. Soc. exp. Biol. Med. 106, 283 (1961).

<sup>5</sup> J. JUNGNER, Scand. J. clin. Lab. Invest. 10, Suppl. 31, 280 (1957).

<sup>6</sup> A. KARMEN, F. WROBLEWSKI, and J. S. LADUE, J. clin. Invest. 34, 126 (1955).

<sup>7</sup> R. ORDELL, Opuscula (Stockholm) 1, 14 (1956).

Electrophoretic separation of protein components of blood serum was performed in cellulose columns (1.5 cm × 50 cm, veronal buffer, pH 8.4,  $I = 0.1$ ) at 5–11°C<sup>8</sup>. For each run, 1.5 ml of serum was used, the current was 30 mA provided by an applied voltage of 340 V, and the duration of runs 16 h. After the completion of electrophoresis, the liquid in the column was displaced at a rate of 10 ml/h in 1.5 ml fractions. The protein concentration of each fraction was estimated by a modified Folin procedure<sup>8</sup>.

The results obtained are illustrated (Figure) with serum samples from man (hepatitis), cow (puerperal paresis), horse (paralytic myoglobinaemia) and pig (hepatosis diabetica). For all four species, both GOT and GPT activities were found in the  $\alpha$ - and  $\beta$ -globulin fractions. Under the experimental conditions used, GOT migrated electrophoretically at a higher rate (man, pig) in comparison with GPT, at about the same rate (cow), or at a slightly lower rate (horse). The human serum analysed contained each of the two transaminases in single fractions, GOT

between the  $\alpha_2$ - and  $\beta$ -globulin fractions and GPT in the  $\beta$ -globulin fraction. A similar result was obtained when electrophoresis was carried out in a phosphate buffer (pH 7.6, 5 mM). The electrophoretic pattern of cow serum showed two peaks of GOT activity, one in the  $\beta$ -globulin region and the other with lower electrophoretic mobility. The main GOT activity of horse serum was found in the  $\beta$ -globulin fraction. In addition, a second peak of low activity was observed in the  $\alpha_1$ -globulin region. GPT activity was found in the  $\alpha_2$ -globulin fraction. Analyses of a series of sera from pigs with hepatosis diabetica revealed that GOT appeared either in one peak in the  $\beta$ -globulin region or in two peaks, one (in the  $\beta$ -globulin region) being common to all samples studied and the other with lower electrophoretic mobility. In addition, certain pig sera gave a peak of low GOT activity in the albumin region. GPT was always found in a single peak migrating close to but slower than GOT.

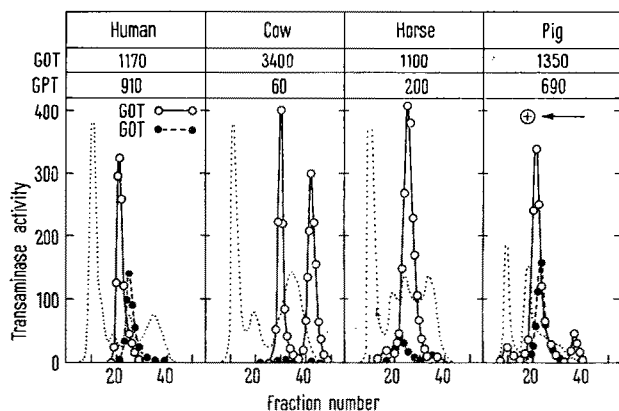
The existence of two fractions with GOT activity in certain mammalian sera may be of importance in clinical studies. If the heterogeneity of this enzyme is actually due to two different molecular forms, these may differ in relative concentration from one disease to another, e.g., in myocardial diseases and hepatic disorders. In addition, it may well be that the two forms have different cellular origin. Our experience with a series of pig sera suggests that differences in clinical course and autopsy findings are reflected in electrophoretic patterns as far as the GOT activity is concerned.

**Zusammenfassung.** Es wird mit Hilfe der Elektrophorese nachgewiesen, dass Glutaminsäure-Oxalacetat-Transaminase im Gegensatz zu Glutaminsäure-Brenztraubensäure-Transaminase in zwei verschiedenen Fraktionen des Blutserums von Kühen und Schweinen vorhanden ist.

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<sup>8</sup> K.-B. AUGUSTINSSON, Acta chem. scand. 13, 571 (1959).



Electrophoretic patterns of sera from man, cow, horse, and pig. Transaminase activity expressed as  $\Delta A_{340} \times 10^3/\text{min/ml}$  fraction. Relative protein contents (...) measured by the Folin colour. Figures on top of each electropherogram refer to the transaminase activity of the original sample (dialysed against the buffer used in electrophoresis).

## Erhöhung der Körpertemperatur von *Periplaneta americana* L. im Verlauf zweier Bakteriosen

Die Angehörigen der Unterklasse Insecta gelten als poikilotherm<sup>1</sup>. Für *Apis mellifica* konnte Esch<sup>2</sup> neuerdings experimentell exakt nachweisen, dass ihr Wärmehaushalt als heterotherm aufzufassen ist. Uns interessierte die grundsätzliche Frage, ob Insekten im Verlauf von Infektionskrankheiten Veränderungen der Körpertemperatur zeigen.

Als Versuchstier wählten wir *Periplaneta americana*, da hier von KÖHLER<sup>3</sup> bereits einschlägige Beobachtungen am gesunden Tier vorliegen. GÖSSWALD<sup>4</sup> berichtete unter anderem über Steigerungen der Körpertemperatur desselben Versuchstieres nach Insektizid-Intoxikationen, wobei beachtet werden muss, dass ein Teil des Vergiftungsablaufes von einer Exzitation des begifteten Tieres begleitet ist. Die von uns verwendeten Erreger *Serratia marcescens* Bizio und *Pseudomonas aeruginosa* Migula (Stamm CCEB 481) werden von KRIEG<sup>5</sup> als fakultative Insektenpathogene kategorisiert. *Serratia marcescens* wurde bereits aus *Periplaneta americana* isoliert<sup>6</sup>, ausser-

dem liegen Infektionsversuche an einer nahen Verwandten, *Blattella germanica* L.<sup>7</sup>, vor. *Pseudomonas aeruginosa* erwies sich für andere Orthopteren bei intracoelomarer Injektion als hochgradig pathogen<sup>8</sup>. In Vorversuchen mit dem letztgenannten Erreger erhielten wir entsprechende Ergebnisse bei *Periplaneta americana*. Nach unseren Beobachtungen kommt es bei keiner der beiden Bakteriosen zu einer Exzitation der Versuchstiere. Vielmehr sind die Krankheitsverläufe unter anderem durch Erschlaffung der Antennen und zunehmende Akinese der Tiere gekennzeichnet. In Spätstadien sahen wir bei der durch *S. mar-*

<sup>1</sup> H. WEBER, *Grundriss der Insektenkunde*, 3. Auflage (1954).

<sup>2</sup> H. ESCH, Z. vgl. Physiol. 43, 305 (1960).

<sup>3</sup> F. KÖHLER, in Vorbereitung.

<sup>4</sup> K. GÖSSWALD, XI. Intern. Entomologenkongress Wien (17. bis 25. 8. 1960).

<sup>5</sup> A. KRIEG, *Grundlagen der Insektenpathologie* (1961).

<sup>6</sup> E. A. STEINHAUS, Hilgardia 28, 351 (1959).

<sup>7</sup> A. M. HEIMPEL und A. S. WEST, Canad. J. Zool. 37, 169 (1959).

<sup>8</sup> G. E. BUCHER und J. M. STEPHENS, Canad. J. Microbiol. 3, 611 (1957).