kung vorgetäuscht wird, waren die Resultate aber grundsätzlich anderer Art: Die Gesamtrate der archenzephalen Gebilde blieb gegenüber den Kontrollen stets gleich oder wurde sogar in unterschiedlichem Masse erhöht (Abb. 4). Eine detaillierte Betrachtung der verschiedenen archenzephalen Gebilde ergab, dass in allen Fermentserien die «starken» archenzephalen Organe (Hirnteile, Augen und Nasen) deutlich vermehrt, während die Zahl der «schwachen» (freie Linsen, Lentoide, Balancer und Epidermisverdickungen) wechselnd beeinflusst, oft auch vermehrt wurden. Wir glauben, nach diesen Befunden von einer echten Erhöhung der archenzephalen Induktionsleistung nach Ribonukleasebehandlung sprechen zu können. Das Vorkommen der spinokaudalen und deuterenzephalen Gebilde lässt keine gesetzmässigen Veränderungen erkennen.

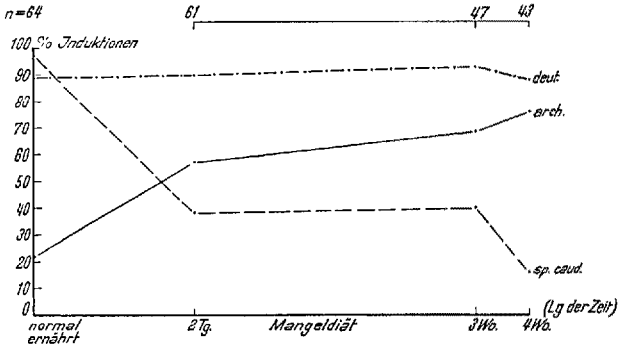

Abb. 3. Induktionsleistungen der Meerschweinchen-Parotis in Abhängigkeit vom Ernährungszustand.

Unsere Befunde scheinen die bisher vorliegenden Vermutungen[1] über die Natur der Induktionsstoffe zu bestätigen. Denaturierung und Abbau gewisser Proteine durch Alkoholbehandlung und Hunger sowie die damit Hand in Hand gehende Auslöschung der spinokaudalen Induktionsleistung sprechen für den Proteincharakter des spinokaudal wirksamen Agens.

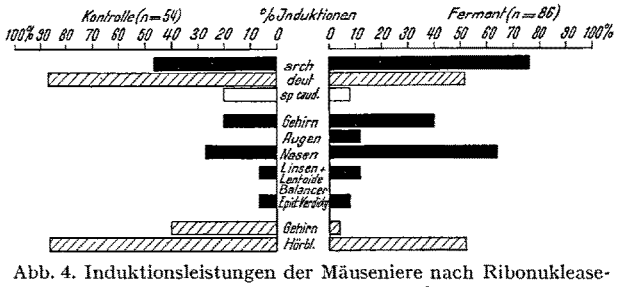

Abb. 4. Induktionsleistungen der Mäuseniere nach Ribonukleasebehandlung (1:2500; 2 h; 40° C).

Das Verhalten der archenzephalen Induktionen im Hunger- und Fermentversuch deutet darauf hin, dass weniger die hochpolymere Ribonukleinsäure als vielmehr deren Spaltprodukte für das archenzephale Induktionsgeschehen von Bedeutung sind; denn durch Hunger werden Abbauprozesse der Ribonukleinsäure eingeleitet, ebenso durch Ribonukleasebehandlung, welche bei unseren Versuchen wahrscheinlich nicht bis zu den letzten, nicht mehr induktionsfähigen Bestandteilen geführt hat. Diese Abbauprodukte könnten auf Grund ihres besseren Diffusionsvermögens vom Induktor in das Reaktionsmaterial die Steigerung der archenzephalen Induktionsleistung in den Fermentserien bewirkt haben.

H. Engländer,
A. Gisela Johnen und W. Vahs

Zoologisches Institut der Universität Köln, den 15. August 1952.

Summary

Various animal tissues were tested for their capabilities as inductors in implantation experiments. After being fixed in alcohol for a short time (few hours), parotis, kidney, and thymus of the guinea pig, as well as the kidney of the white mouse (Series I), proved to be specifically deuterencephalic-spinocaudally acting inductors, whereas the liver and heart of the guinea pig, and the liver of mouse and Triturus (Series II), showed themselves as specifically archencephalic-deuterencephalic inductors.

If the affection by alcohol is prolonged (up to 4 weeks), the deuterencephalic-spinocaudal effect is gradually lost in series I, while new archencephalic structures come into existence.

The tissues of series II, however, retain their archencephalic-deuterencephalic effect. The action of the tissues tested, except that of the heart muscle, changes in the same way when the animals are subjected to a starvation diet for several weeks. The archencephalic rate of induction increases while the spinocaudal rate decreases in proportion. In all the tissues tested, the capacity of archencephalic induction can be increased by treatment with ribonuclease.

[1] F. E. Lehmann, *Einführung in die physiologische Embryologie* (Verlag Birkhäuser, Basel 1945), S. 319 ff. – S. Toivonen, Rev. suisse Zool. *57*, 41 (1950). – T. Kuusi, Ann. Zool. Soc. zool.-bot. Fenn. «Vanamo» *14*, 4, 1 1951).

Dynamic State and Half Life Time of Human Serum Cholinesterase

An important biological concept evolved in recent years is that of the dynamic state of the body constituents. Formerly, for example, for one of these, the body proteins, semi-static stores were implied from which proteins were removed by catabolism and to which the anabolism of the body supplied replacements. Actually, Schoenheimer[1] has demonstrated, using the isotope technique, that there is a regular, continuous, dynamic interchange of the body elements in which there is concurrent synthesis and degradation.

One method employed illustrates the general isotope approach: an amino acid, for example, glycine, labeled with radioactive N^{15}, is administered to the animal. Various proteins are then collected at periodic intervals, isolated, and quantitatively studied as to isotope concentration per unit weight of protein. The peak for the incorporation of the glycine is reached shortly following cessation of administration, following which a decline occurs. This corresponds to the disappearance through degradation of the isotope labeled protein molecules. The rate of decline indicates a constant replacement of the labeled with newly synthesized unlabeled molecules. This is illustrated in Figure 1. It is customary to denote the average time of replacement of these molecules as the half life time of the particular protein being studied.

We are interested in an enzyme, pseudocholinesterase, which is found in human serum, and which we have

[1] R. Schoenheimer, *The Dynamic State of the Body Constituents* (Harvard University Press Cambridge, Mass., 1942).

reported[1] to have some quantitative relationship to serum albumin in the human. If we could relate the rate of synthesis of this enzyme to that observed for albumin by the isotope method, we would have a further means of understanding the relationship of the two. Until now, the semi-static concept of the state of the pseudocholinesterase has assumed that it is synthesized in the liver and is then transferred to the blood stream as needed to maintain a constant blood level.

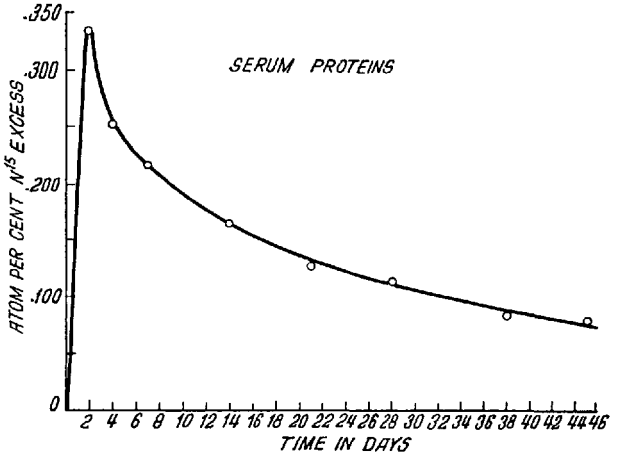

Fig. 1.—N^{15} concentration in serum proteins after feeding N^{15} labeled glycine for 2 days. The slope of the curve from the peak concentration of the labeled protein to its minimum value indicates the rate of destruction of serum protein. [After I. M. LONDON, in *Plasma Proteins*, ed. by J. B. YOUMANS (Thomas, Springfield, 1950)].

For the present it is impossible to utilize the isotope method for serum cholinesterase, since the procedure is of value only if the enzyme may be purified considerably. This has as yet not been accomplished with any success. We have, however, arrived at another method for determining the same information so dramatically illustrable through the isotope procedure. It has been demonstrated that serum cholinesterase is inhibited by a number of chemical substances including Di-isopropyl Florophosphate (D.F.P.), a relatively irreversible[2] inhibitor. *In vivo*, the administration of this drug is followed by the rapid disappearance, amongst other things, of serum cholinesterase followed by its immediate regeneration[3]. The rate of this regeneration indicates the same information as to the dynamic state of this particular protein which the isotope has given for other tissue elements. Because of a constant level of this enzyme in the human over a long period of time, the rate of reappearance of the enzyme would actually indicate the time and quantity of the regular synthesis of the molecules. Since the experiment in humans offers some difficulty, it has been possible to secure data for this regeneration from other sources and this is presented in Figure 2.

Medhod. The data of four groups of investigators have been examined statistically. Series number one[4] consists of 13 normal individuals who received 2 mg of D.F.P. in peanut oil intramuscularly. Series number two[1] consists of 35 subjects, 25 of whom were normal convalescents and ten with myasthenia gravis. 5 tenths of 3 mg of D.F.P. were administered intramuscularly or 0·5 to 2 mg in aqueous solution was given intra-arterially. Series number three[2] consists of 8 normal subjects with determinations on the 4th, 8th, and 15th day and 9 subjects on the first day. D.F.P. was administered through vapor inhalation. Series number four[3] consists of 6 subjects who were given 2 to 3 mg of D.F.P. intramuscularly in peanut oil. In all of these the standard WARBURG technique was employed to determine serum cholinesterase. Determinations were made immediately following cessation of D.F.P. administration at which initial point the serum cholinesterase had disappeared almost completely from the serum. For the data of GROB *et al.*[4] and MAZUR and BODANSKY[2], the curves are plotted from tables included in their papers. The data of WESCOE *et al.*[5] consists of replotting the curve included in their paper so that the values are determined as percent regeneration rather than actuel serum cholinesterase activity readings. The curve obtained from

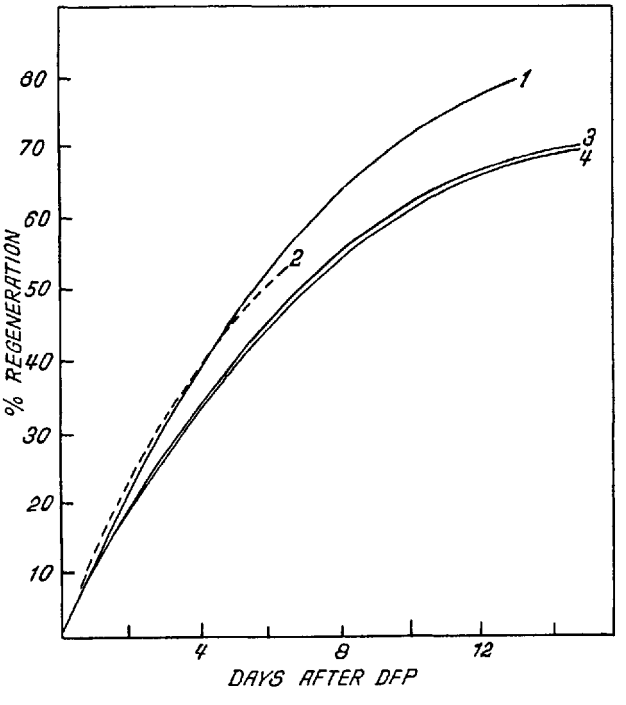

Fig. 2.—Regeneration of human serum cholinesterase following the administration of D.F.P. Curve No. 1 from the data of WESCOE *et al.*[5]. Curve No. 2 from the data of LILIENTHAL *et al.*[1]. Curve No. 3 from the data of MAZUR and BODANSKY[2]. Curve No. 4 from the data of COMROE *et al.*[3].

[1] M. G. LEVINE and R. E. HOYT, Science *111*, 286 (1950). – M. G. LEVINE and A. A. SURAN, Proc. Sco. Exp. Biol. Med. *79*, 686 (1952).

[2] A. MAZUR and O. BODANSKY, J. Biol. Chem. *163*, 261 (1946).

[3] A. MAZUR and O. BODANSKY, J. Biol. Chem. *163*, 261 (1946). – W. C. WESCOE, C. H. CARLTON, W. F. RIKER, and I. C. LITT, Amer. J. Phys. *149*, 549 (1947). – D. GROB, J. L. LILIENTHAL, A. M. HARVEY, and B. F. JONES, Bull. Johns Hopkins Hosp. *81*, 217 (1947).

[4] W. C. WESCOE, C. H. CARLTON, W. F. RIKER, and I. C. LITT, Amer. J. Phys. *149*, 549 (1947).

[1] D. GROB, J. L. LILIENTHAL, A. M. HARVEY, and B. F. JONES, Bull. Johns Hopkins Hosp. *81*, 217 (1947).

[2] A. MAZUR and O. BODANSKY, J. Biol. Chem. *163*, 261 (1946).

[3] J. H. COMROE, J. TODD, and G. B. KOELLE, J. Pharm. Exp. Ther. *87*, 281 (1946).

[4] D. GROB, J. L. LILIENTHAL, A. M. HARVEY, and B. F. JONES, Bull. Johns Hopkins Hosp. *81*, 217 (1947).

[5] W. C. WESCOE, C. H. CARLTON, W. F. RIKER, and I. C. LITT, Amer. J. Phys. *149*, 549 (1947).

the data of COMROE and his co-workers[1] has been constructed from Table 3 taken from that paper.

From Figure 2 we observe the following: There is a relatively close resemblance between the data of the four separate experimental groups. The curve for the regeneration is exponential and similar to those obtained through isotope studies. It may be concluded, therefore, that this enzyme exists in the body in the same dynamic state as, for example, serum albumin. We may from these curves also obtain the half life time of the enzyme, approximately 6–7 days. This may be compared with the reported[2] half life time value for serum albumin of 20 days obtained for normal man. Actually, the half life time obtained with the D.F.P. procedures is not strictly comparable to that gotten from the isotope method. We are at present attempting a mathematical formulation which will make it possible to translate one into the other. This paper has been presented, since it offers a new method for determining the dynamic state of an enzyme. The data for serum cholinesterase has been presented above, but the procedure may be used generally for such determinations with other enzymes. With D.F.P., important information may be obtained not only for serum cholinesterase, but for true cholinesterase as it occurs in many tissues.

M. G. LEVINE

Kabat-Kaiser Institute, Vallejo, California, November 29, 1952.

Zusammenfassung

Eine Methode wird dargestellt, mittels welcher das dynamische Verhalten und die Halblebenszeit eines Enzyms berechnet werden kann. Es wird ein Inhibitor hinzugefügt und nachher die Regeneration, *in vivo*, bestimmt. Das Enzym ist «Pseudocholinesterase» und der Inhibitor ist Di-isopropyl-Floro-phosphate.

[1] J. H. COMROE, J. TODD, and G. B. KOELLE, J. Pharm. Exp. Ther. *87*, 281 (1946).

[2] I. M. LONDON, D. SHEMIN, R. WEST, and D. RITTENBERGER, J. Biol. Chem. *179*, 463 (1949).

Host Resistance to Tumor Implantation, its Alteration and Relationship to the Individual Metabolic Pattern[1]

We have been working in this laboratory for several years with a transplantable fibrosarcoma, the implantation incidence of which was relatively low in a closely-bred strain of SHERMAN rats. An attempt to explain this low incidence led to the consideration of a number of possible causes.

If, for any reason, transplanted fragments do not possess a comparable potential of active cells, due to variation within the tumor stock itself, or if certain variables in the technique of grafting are not adequately controlled, one may expect a lowered incidence. On the other hand, it is plausible to assume, the tumor stock and variations in technique being controlled, that some individuals are more susceptible than others to a tumor implant. Under such conditions, one should be able, by challenging with grafts, to segregate tumor-resistant from tumor-susceptible animals. With such groups available, a new approach to the problem of the nature of resistance to cancer becomes possible through a comparative study of the metabolism of these respective groups of animals.

Our first efforts were, therefore, concerned with establishing a reliable tumor transplantation technique and eliminating possible variation within the tumor stock. An early study[1] showed that the age of the host bearing a stock tumor had a distinct effect on viability of the tumor. It was demonstrated that when rats were implanted with tumors at the age of 10–15 days, the tumors which developed within 2–3 weeks were better for transplantation purposes than those grown in older animals. Such tumor stocks from young animals were subsequently used. It was also shown that all portions of such tumors are equally viable[2]. By propagating the tumor in young animals at regular intervals, and utilizing stocks of the proper age, variations within the tumor stock have been definitely controlled.

The methods for handling the tumor during the process of grafting have likewise been carefully standardized. Grafting is carried out in a constant-temperature room at 24°C, which was shown to be more favorable to viability than 37°C. Graft size, within a certain range, is a critical factor, but it can be controlled so that implantation incidence following replicate grafting with fragments from the same or similar stock tumors in the same animals does not differ significantly[3]. As a matter of fact, segregation of resistant from susceptible animals has been achieved by challenging with quadruple grafts.

Other studies, in which standard techniques and tumor stocks were used, showed that the resistance to implantation of a significant number of individuals within an experimental group may be affected by specific factors and that it can be altered by nutritional means. Age of individuals receiving grafts is a determining factor[4]. Dietary supplementation with pyridoxine favors implantation[5], as does supplementary administration of thymic extracts[6]. Phenylalanine supplements, however, lowers incidence of successful implants[7], while riboflavin has no effect on incidence, but does affect growth of the tumor after implantation[8].

Having shown that resistant animals can be segregated from susceptible ones and that dietary supplementation can alter resistance, it is of interest to know what may be the physiological basis for the phenomenon of resistance.

It would seem that implantation and growth of tumors, being subject to nutritional control, constitutes another case of what is characterized as "genetotrophic" diseases by R. J. WILLIAMS *et al.*[9]. Individual and strain variation in regard to tumor susceptibility may simply be an expression of metabolic pattern variation. Quantitative analytical determination of the constituents of representative body fluids, such as urine, have revealed that

[1] Presented at the Second International Congress for Biochemistry (Section on Cancer), Paris, July 1952. Aided by a grant from the Damon Runyon Memorial Fund.

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[2] J. B. LOEFER, R. B. MEFFERD, Jr., and R. M. NETTLETON, Jr., Texas Rep. Biol. Med. *10*, 598 (1952).

[3] R. B. MEFFERD, Jr. and J. B. LOEFER, Texas Rep. Biol. Med. *10*, 608 (1952). – J. B. LOEFER and R. B. MEFFERD, Jr., Cancer *1953* (in press).

[4] J. B. LOEFER and N. G. GILLES, Cancer *4*, 1259 (1951). – J. B. LOEFER, Cancer *5*, 163 (1952).

[5] J. B. LOEFER, Can. Res. *11*, 481 (1951).

[6] J. B. LOEFER and N. G. GILLES, Texas Rep. Biol. Med. *9*, 571 (1951).

[7] J. B. LOEFER and R. B. MEFFERD, Jr., Texas Rep. Biol. Med. *10*, 614 (1952).

[8] R. B. MEFFERD Jr. and J. B. LOEFER, Texas Rep. Biol. Med. *10*, 619 (1952).

[9] R. J. WILLIAMS *et al.*, Biochem. Inst. Studies. IV. Univ. Texas Publ. 5109, 205 pp. (1951).