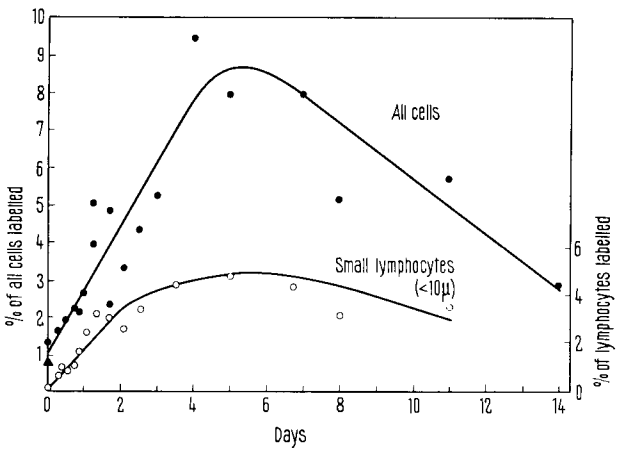
increases at a rate of 1.9% per day, reaching a maximum of 8.5% by the 4th to 6th day. This increase in the proportion of labeled cells is attributable to both large mononuclear cells and small lymphocytes. The latter increase from 0.1% at zero time to 4.9% by the 4th day.

Peritoneal large mononuclear cells are heavily labeled 20 min after injection of ^{3}HTdR (median grain count 49, range 20–60), remain at this level through the 4th day (44 grains/cell), then fall to 13 grains/cell (range 7–34) by the 14th day. Small lymphocytes in both blood and peritoneal fluid are also heavily labeled on the 1st day (median grain count 35 for peritoneal lymphocytes, 50 for blood lymphocytes) but the range is similar in both cases (20–60) and the means are not statistically different.

These data are consistent with the view that the labeled small lymphocytes which appear in peritoneal fluid originate in the blood. Although some lymphocytes might originate from division of large mononuclear cells within the peritoneum, the grain counts and, particularly, the paucity of mitotic figures in the large mononuclear cell population indicate that this source is not quantitatively important.

The peritoneal cavity of a 200 g rat contains approximately 12.5×10^6 cells of which 60% (7.6×10^6) are small lymphocytes[4]. The proportion of labeled small lymphocytes increases at a rate of 1.7% per day during the first 2 days (Figure), so (neglecting exit of labeled cells) about 6.5×10^4 labeled lymphocytes join the population each day, or 2.7×10^3 labeled cells/h. Since less than 2% of the small lymphocytes in blood are labeled during this interval[6], the total influx of small lymphocytes (labeled plus unlabeled) into the peritoneal fluid may be as much as 50-fold greater, or 1.4×10^5 cells/h. Since the total number of peritoneal lymphocytes remains constant, the same number of cells must leave the peritoneal fluid (or be destroyed within it) as enter.



Percent of labeled cells in rat peritoneal fluid after a single i.v. injection of ^{3}H-TdR. Each point was obtained by counting 5000 cells from a pooled sample obtained from 2 or 3 rats. The labeling after a single i.p. injection of ^{3}H-TdR is also shown (▲). Points on the lymphocyte curve represent a 3-point moving average calculated from the actual data.

If we ignore the movement of cells out of the population and assume that peritoneal small lymphocytes are homogenous with respect to residence-time within the peritoneal fluid, the turnover time (time for complete replacement) for these cells is $7.6 \times 10^6/(6.5 \times 10^4)(50) = 23.4$ days. However, many lymphocyte compartments contain a mixture of short-lived and long-lived cells and if this is also true for the peritoneal fluid the calculated turnover time is merely the mean for 2 (or more) populations. Obviously, the calculated value cannot be taken too seriously until this matter can be resolved, since the calculation will be greatly influenced by the presence of even a small number of very short-lived or very long-lived cells[9].

Zusammenfassung. Zellkinetische Untersuchungen an Ratten nach Injektion von H^3-Thymidin ergaben eine rasche Markierung der grossen mononukleären Zellen innerhalb von 20 Min und eine langsamere Markierung der kleinen Lymphozyten, für welche eine Turnover-Zeit von 23,4 Tagen errechnet wurde. Stündlich gelangen ca. 10^5 kleine Lymphozyten in die Bauchhöhlenflüssigkeit.

J. W. Harris, J. M. Yoffey[9] and N. P. Allen

Laboratory of Radiobiology, University of California Medical Center, San Francisco (California 94122, USA), 13 January 1970.

[9] This research was performed under the auspices of the US Atomic Energy Commission. Prof. Yoffey's present address is: Department of Anatomy, Hadassah Medical School, Box 1172, Jerusalem (Israel).

Calf Thymus Fractions: Enhancement and Suppression of Immunocompetent Cells in Neonatal Mice

The functional significance of the thymus has been an enigma for many years. Recent studies have indicated that the thymus may function, at least in part, as a source of humoral substances necessary for development of lymphoid tissue and immunological integrity[1]. Earlier studies indicated that thymus extracts may induce lympho cytopoesis and increased lymphoid tissue size[2,3]. More recent reports have indicated that thymus extracts can also prevent wasting disease and fatal virus infections in thymectomized mice[4–6].

A fraction derived from calf thymus, termed thymosin, was prepared by Goldstein, Slater and White[7] in 1966. This factor could stimulate lymphoid tissue hyperplasia and DNA, RNA and protein synthesis in vivo. In 1967, Hand, Castor and Luckey[8] prepared a basic protein from calf thymus extracts. This substance stimulated lymphocytopoesis and lymphoid tissue hyperplasia in mice when administered in microgram quantities. Law and Agnew[9], and Law, Goldstein and White[10] reported that thymosin could restore immunologic reactivity of spleen cells of thymectomized mice. Recently, Trainin, Small and Globerson[11] demonstrated in an in vitro system that a thymus extract could restore immunocompetence to spleen cells from thymectomized mice. However, relatively large amounts of their substance were necessary. We now describe the effects of purified

calf thymus extracts which, in microgram quantities, can either stimulate or inhibit development of immunocompetence in neonatal mice when administered on the day of birth.

Experimental. The calf thymus extracts were prepared by fractionation procedures described previously[8]. Fresh pooled calf thymus glands were homogenized in 0.85% sterile saline. After centrifugation, the debris was discarded and the crude saline extract (CSE) was precipitated with 20% ammonium sulfate. The resulting precipitate was dissolved in distilled water and treated with absolute methenol at a ratio of 3:1. The resulting precipitate was recovered and dissolved in distilled water. This was considered the partially purified extract (PPE). For further fractionation, this material was subjected to polyacrylamide gel electrophoresis, resulting in a number of distinct fractions. Fraction B-C, previously found to contain a lymphoid cell stimulator, and fraction D-E, found to oontain an inhibitory material, were used for the immunologic studies described here.

Non-inbred pregnant ICR mice were observed at approximately 8-h intervals and the time of litter delivery and number was recorded. Litters were injected i.p. within 12 h of birth with either CSE, PPE, or fractions B-C or D-E. Control litters were injected with saline only. Mice from each group were challenged with 0.1 ml of a 50% suspension of sheep red blood cells (SRBC) after treatment at birth or 1, 2, 3, or 4 weeks thereafter.

The immune response was assayed 4 days after immunization by means of the haemolytic immunoplaque assay, which permits enumeration of antibody plaque-forming cells (PFCs)[12].

Results and discussion. As can be seen from the Table, control mice injected with SRBC had only the anticipated progressive increase in the number of PFCs with age. Although new-born and 1-week-old animals had few plaque-forming cells after challenge, by the 2nd to 4th week of life these animals responded with 40,000 to 90,000 PFCs per spleen. The crude saline extract did not markedly affect antibody-forming potential in animals of this age group.

Inoculation of mice at birth with PPE resulted in a significant enhancement of the number of PFCs (Table). Immunization of these mice at 1 week of age resulted in about 4 times as many PFCs as controls. Immunization at 4 weeks of age resulted in almost twice as many PFCs as controls when calculated per whole spleen, and over 10 times as many when calculated per million splenic leucocytes. A PPE prepared from liver and kidney was without stimulatory effect when tested under similar conditions.

Injection of mice with 3 μg of purified gel fraction A-B resulted in an enhancement of PFC development during the first weeks of life. There was a 15–40-fold enhancement of the number of PFCs from mice following challenge at 1 week of age (Table). There was a 20–40% enhancement at 2 and 4 weeks of age.

In contrast, fraction D-E markedly suppressed development of immunocompetence. Injection of this material at birth resulted in a 90–95% depression in the number of PFCs in the spleens of animals challenged during the first 2 weeks of life (Table). At the 4th week, injected animals had essentially the same number of PFCs per spleen as controls.

The results of these studies indicate that fractions derived from calf thymus glands may influence development of immunocompetence of neonatal mice. Crude saline extracts had little effect, whereas a partially purified extract stimulated an enhancement of PFC development. The most marked enhancing effect occurred with the electrophoretic fraction B-C, previously found to stimulate lymphocytopoesis. This fraction resulted in a 15–40-fold enhancement in the number of PFCs during the first 2 weeks of life. A second gel fraction, designated D-E and previously found to inhibit lymphocytopoesis, caused a marked diminution of the PFC response.

Effect of crude and prepared calf thymus extracts and fractions on development of immunocompetence to sheep erythrocytes when injected into mice at birth and challenged with antigen at 0, 7, 14, or 28 days of age

Substance injected at birth[a]	Age at time of PFC determination (days)			
	4	11	18	32
Plaque-forming cells per spleen				
None	18	5,100	40,300	94,000
CSE	18	4,480	52,000	72,000
PPE	40	20,000	62,500	145,000
Fraction B-C	26	75,500	50,000	140,000
Fraction D-E	25	109	16,000	94,500
Plaque-forming cells per 10^6 splenocytes				
None	3	54	210	331
CSE	2	60	220	380
PPE	8	140	245	4,250
Fraction B-C	2	2,000	306	585
Fraction D-E	3	3	32	521

[a] Mice injected on day of birth with 15 μg of crude saline extract (CSE) or partially purified extract (PPE) or with 3 μg of gel electrophoresis fraction A-B or C-D and then challenged i.p. with 0.1 ml 50% SRBC at indicated age. Average number of PFCs for 5 or more mice determined 4 days after immunization.

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The mechanism whereby PPE and fraction B-C stimulate development of antibody-forming cells is not known. It is possible that these materials affect the pool of precursor stem cells, which were already present and developing in untreated animals, or provide factors necessary for more rapid maturation and proliferation of all cells involved in immunogenesis.

The relationship between the fractions described here and those reported by others is not clear. The purified fractions tested in this study are effective when administered at dose levels much less than those reported by others. Nevertheless, it seems plausible that there may be close biochemical and biological relationships among all the fractions and extracts studied by various investigators[13].

Zusammenfassung. Nachweis, dass eine aus Kälber-Thymus isolierte Fraktion auf die Ausbildung von Hämolysin produzierenden Zellen in neugeborenen Mäusen einen stimulierenden Einfluss haben kann.

T. Hand, W. S. Ceglowski and H. Friedman

Departments of Microbiology, Temple University School of Medicine and Albert Einstein Medical Center, Philadelphia (Pennsylvania 19140, USA), 15 December 1969.

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Die Wirkung von Vasopressin und Oxytocin auf das Nervensystem von Insekten

Zahlreiche Autoren[1–6] konnten in den letzten Jahren den Nachweis für die Wirksamkeit verschiedener Neurohormone sowohl von Wirbellosen als auch von Wirbeltieren auf die spontane Impulsaktivität zentralnervöser und peripherer Nervenstrukturen erbringen. Da es sich bei den bisher bekannten Neurohormonen der Wirbellosen wahrscheinlich um Oligopeptide mit Disulfid-Brücken handelt[2,7], ergibt sich auf Grund der zu vermutenden Strukturähnlichkeiten bei weitgehend gleichartig funktionellem Verhalten dieser Substanzen die Frage, ob und in welchem Masse die Funktionsweise des Nervensystems von Insekten durch die körperfremden Neurohormone Vasopressin und Oxytocin beeinflusst werden kann.

Als Versuchstiere dienten adulte amerikanische Küchenschaben (*Periplaneta americana* L.). Die Impulsaktivitäten wurden vom Konnektiv zwischen dem 5. und 6. Abdominalganglion sowie vom efferenten und afferenten Cercalnerven abgeleitet. Die Substanzen (Arginin-Vasopressin und synthetisches Oxytocin) wurden in Insektenringer nach Roeder (NaCl 9,0; KCl 0,2; CaCl 0,2; Glucose 4,0 g; 10 ml Phosphatpuffer pH 7,2 ad 1000 ml Aqua bidest.) gelöst auf das 6. Abdominalganglion aufgebracht beziehungsweise mittels Kanüle in den gespaltenen Cercus appliziert. Die Analyse der Impulsaktivität erfolgte mit dem Computer CAT 400 C.

Vasopressin hat excitatorische Wirkung und verursacht in einer Konzentration von 0,1 µE/ml eine Erhöhung der Impulsfrequenz um 100 bis 200%, verbunden mit verstärkter Salvenbildung. Höhere Konzentrationen (20 mE/ml und 250 mE/ml) führen initial zu einer Frequenzsenkung. Dabei nimmt der Anteil kurzer Intervalle für die Dauer von 30 min zu.

Oxytocin bewirkt in allen untersuchten Abschnitten des Nervensystems eine Frequenzsenkung um 50%. Die stärkste inhibitorische Wirkung wird bei einer Konzentration von 2 µE/ml beobachtet. Dabei nimmt die Zahl kurzer Intervalle, im Gegensatz zur Vasopressinwirkung, stark ab.

Bezüglich der Impulsaktivität nach Wirkstoffgabe bestehen keine deutlichen Unterschiede zwischen efferenten und afferenten Nervenbahnen. Damit erweisen sich die Neurohormone Vasopressin und Oxytocin an Insektennerven als antagonistisch wirksame Substanzen mit umgekehrt konzentrationsabhängigem Verhalten. Es ist zu vermuten, dass beide Neurohormone die Membrandurch-

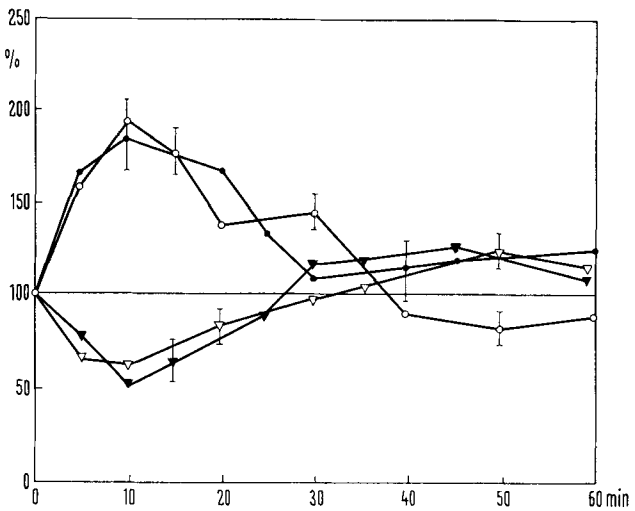

Fig. 1. Veränderungen der Impulsfrequenz des efferenten und afferenten Cercalnerven nach Vasopressin- und Oxytocingaben. Vasopressin: ○, efferenter Cercalnerv; ●, afferenter Cercalnerv. Oxytocin: ▽, efferenter Cercalnerv; ▼, afferenter Cercalnerv.

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