(females) were treated similarly but sacrificed 1 h after fracture; group 4 (males) and group 9 (females) were sacrificed 3 h, and groups 5 (males) and 10 (females) were sacrificed 6 h after tibial fracture. At sacrifice, the adrenals were also excised and weighed.

Table II shows that the mean baseline level of plasma corticosterone was identical in male and female gonadectomized rats. $^1/_2$ h after tibial fracture no sex difference was observed in the mean plasma corticosterone levels. At 1 h and thereafter, however, mean levels were significantly greater in the female than in the male rats. The lesser response of the male castrated rats is reflected also in the adrenal weights.

The above results confirm the findings of others (COURRIER et al.[10], KITAI[4]) regarding the greater sensitivity of the female adrenal gland to exogenous and perhaps also endogenous ACTH. This difference, however, was found in mature rats only.

The greater size of the female adrenal gland (HATAI[11,12]) has suggested the existence of a difference between male and female adrenal function also. Thus, ovariectomy resulted in adrenal atrophy (ANDERSEN and KENNEDY[13], FREUDENBERGER and HASHIMOTO[14]) and replacement with estrogen in such animals prevented this (CARTER[15]). While studies on plasma corticosterone levels had been reported for intact male and female rats[4], and had shown greater levels in the females, no such studies have been performed in gonadectomized rats. In the present study, there was no evidence of a sex difference in the baseline level of plasma corticosterone of non-stressed, gonadectomized rats. This agrees with the in vitro findings of TROOP and POSSANZA[5] that male and female rat adrenals produced similar quantities of corticoid in incubation for 2 h. Our findings further suggest that gonadectomy does not affect the inherent sex difference in terms of plasma corticosterone in response to stress. This is in accordance with the findings on intact males and females made by KITAI[4] and in castrated males and spayed females in in vitro studies after the addition of ACTH (TROOP and POSSANZA[5]).

Thus, the responsiveness of the adrenal gland is not dependent on gonadal function since if it were, gonadectomy would affect this pattern. Under the circumstances the best interpretation for our findings is KITAI's[4] concept of greater responsiveness of the female adrenal gland to ACTH.

Table II. Plasma corticosterone (μg/100 ml)

Time of sacrifice (h)	Group No.	Male rats	Group No.	Female rats
0	1	9.1[a] ± 1.35	6	8.0 ± 1.50
0.5	2	56.8 ± 2.37	7	62.8 ± 4.30
1	3	60.2[c] ± 6.34	8	86.2[d] ± 2.53
3	4	57.3[e] ± 3.70	9	78.5[f] ± 3.35
6	5	53.5[g] ± 3.35	10	73.5[h] ± 2.43
		Adrenal weight (mg)		
0	1	53.9 ± 1.45	6	56.6 ± 1.88
0.5	2	44.5[i] ± 1.53	7	55.0[j] ± 1.84
1	3	43.5[k] ± 1.03	8	52.8[l] ± 2.92
3	4	47.9[m] ± 1.11	9	55.3[n] ± 1.45
6	5	48.0[o] ± 1.49	10	56.8[p] ± 2.56

[a] Mean ± S.E.M. [c] vs [d], [k] vs [l], [o] vs [p] = $p < 0.01$; [e] vs [f], [g] vs [h] [i] vs [j], [m] vs [n] = $p < 0.001$.

Table II. Plasma corticosterone levels and adrenal weights of male and female rats that were gonadectomized shortly after weaning. Groups 1 and 6 served as unstressed controls. Groups 2–5 and 7–10 were subjected to bilateral tibial and fibular fracture and sacrificed at the times indicated.

Zusammenfassung. Injektion von 2 IE ACTH in hypophysektomierte unreife Ratten beiderlei Geschlechts: kein Geschlechtsunterschied des Corticosteron-Plasma-Spiegels (CPS). Gleiche Behandlung von weiblichen hypophysektomierten, geschlechtsreifen Ratten: $^1/_2$ h nach der Injektion bereits höherer CPS. Kastrierte, weibliche unbelastete Ratten zeigten keinen Unterschied in der Höhe des CPS gegenüber kastrierten, unbelasteten männlichen Ratten. Nebennieren-Belastung solcher weiblicher Ratten durch bilaterale Fraktur der Tibiae und Fibulae: höherer CPS 1, 3 und 6 h nach Fraktur. Die Befunde deuten darauf hin, dass die weibliche Nebenniere eine grössere Empfindlichkeit für ACTH-Wirkung hat.

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[10] R. COURRIER, R. GUILLEMIN, A. COLONGE, and E. SAKIZ, J. Acad. Sci. *252*, 3520 (1961).
[11] S. HATAI, Am. J. Anat. *15*, 119 (1913).
[12] S. HATAI, Anat. Record *8*, 511 (1914).
[13] D. H. ANDERSEN and H. S. KENNEDY, J. Physiol., Lond. *79*, 1 (1933).
[14] C. B. FREUDENBERGER and E. I. HASHIMOTO, Proc. Soc. exp. Biol. Med. *41*, 532 (1939).
[15] S. B. CARTER, J. Endocr. *13*, 150 (1956).

DISPUTANDUM

The Influence of the Thymus on Radiogold Clearance

MILLER was the first to point out the primary role of the thymus in immunity[1]. Numerous experiments have since confirmed that thymectomy of new-born mice or thymectomy of adult animals combined with whole-body irradiation or cytostatic agents results in immunological deficiency[2]. On the other hand, the connection between thymus and phagocytosis is obscure. Therefore, it is not known whether phagocytosis is caused by a central ner-

[1] J. F. A. P. MILLER, Lancet *ii*, 748 (1961).
[2] J. F. A. P. MILLER and P. DUKOR, *Die Biologie des Thymus* (S. Karger-Verlag, Basel-New York 1964).

vous or humoral mechanism, or whether it is autonomous[3]. The present investigation was undertaken to ascertain whether the phagocytic activity of the reticulo-endothelial system is influenced by the thymus.

The reticulo-endothelial system is able to store formed organic or inorganic substances taken up from the blood stream[4]. Phagocytosis can therefore be evaluated by determination of the blood clearance of radioactive colloids[5]. The blood clearance is an exponential function of time. The $K = \log C_1 - \log C_2/t_2 - t_1$, phagocytic index is a measure of the phagocytic activity of the RES[6].

The experiments were performed with 90 male 5-week-old Swiss mice, divided into 2 groups. In both groups, 15 mice were thymectomized, 15 sham thymectomized and 15 served as controls[7]. 7 days later, all 45 mice of the second group were irradiated with 350 R (200 kV, 20 mA, FHD 50 cm) in order to induce immuno-suppression. On the 14th post-operative day, the clearance of 120 μg of colloidal radiogold (size 250 Å) was measured with a scintillation counter over the neck[8]. The phagocytic index was determined between 3 and 6 min after the intravenous injection of radiogold.

Results. The phagocytic index of the thymectomized mice is $K = 0.400 \pm 0.05$, which is lower than the index of the controls (Table). This difference is statistically significant at the 5% level. This means that the radiogold clearance of the animals thymectomized 2 weeks previously is reduced in comparison to the sham thymectomized and the control mice. On the other hand, the whole-body irradiation with 350 R has no influence on the clearance. The phagocytic index corresponds to that of the non-irradiated mice.

The phagocytic index K and standard deviation 14 days after thymectomy or sham thymectomy

Thymectomy		0.400 ± 0.051
Sham thymectomy		0.450 ± 0.064
Control group		0.446 ± 0.071
Thymectomy	+ 350 R	0.404 ± 0.049
Sham thymectomy	+ 350 R	0.446 ± 0.073
Control group	+ 350 R	0.450 ± 0.067

Discussion. The reduced radiogold clearance of the adult thymectomized mice may be the result of a lower colloid storage capacity of the organism. It therefore seems that the phagocytic activity is lower after thymectomy. Since radiation damage of the lymphatic tissue does not reduce radiogold clearance, one may assume that this effect on phagocytic activity is not due to delayed immune response but caused by the loss of the thymus. We can therefore assume that the thymus influences both immunity and phagocytosis. Each system, however, is a functional unit per se which is controlled cellularly or humorally by the thymus[9].

Zusammenfassung. Die Radiogoldclearance thymektomierter erwachsener Mäuse ist gegenüber scheinthymektomierten bzw. den Kontrolltieren vermindert. Eine zusätzliche Immundepression durch Ganzkörperbestrahlung mit 350 R führt gegenüber den einzelnen Vergleichsgruppen zu keiner Änderung der Radiogoldclearance. Daraus kann mit aller Vorsicht geschlossen werden, dass der Thymus sowohl die Immunreaktionen als auch die Phagozytosefunktion als übergeordnetes Zentrum steuert. Jedes System scheint aber für sich eine funktionelle Einheit zu bilden.

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[3] B. N. Halpern, J. Pharm. Pharmac. *11*, 321 (1959).
[4] B. N. Halpern, G. Biozzi, and B. Benacerraf, Am. J. Physiol. *189*, 520 (1957).
[5] E. L. Dobson, *Physiopathology of the RES* (Pergamon Press, Oxford 1957).
[6] G. Biozzi, B. N. Halpern, and C. Stiffel, *Radioactive Isotope in Klinik und Forschung* III (Urban und Schwarzenberg, München-Berlin 1958).
[7] We thank Dr. P. Dukor for introducing us to the operation technique.
[8] R. Fridrich and M. Schäfer, Experientia *21*, 40 (1965).
[9] The experiments were supported by a grant from the Swiss National Fund.

PRO EXPERIMENTIS

Fluorometrische Bestimmung von Mikromengen Calcium in Muskelgewebe

Die komplexometrische Bestimmung von Calcium in biologischem Material ist durch Verwendung des Indikators Calcein (Fluoresceinbismethyliminodiessigsäure), der in stark alkalischem Milieu (pH ~ 13) mit Ca^{2+} einen fluoreszierenden Komplex bildet, und durch automatische Registrierung und graphische Auswertung der Titrationskurven bereits beträchtlich verbessert worden[1]. Ausgehend von der Beobachtung, dass unter bestimmten Bedingungen eine direkte Abhängigkeit der Fluoreszenzintensität des Ca-Calcein-Komplexes von der Ca-Konzentration besteht[2], wurde nun eine einfachere und noch empfindlichere fluorometrische Ca-Bestimmungsmethode entwickelt.

Apparaturen. Gemessen wurde (a) mit einem Spektralfluorometer (Zeiss ZFM 4C mit 2 Monochromatoren, Xenonlampe XBO 450 W/P) gegen den Zeiss-Standard

[1] P. Cartier und J. Clément-Métral, Clinica chim. Acta *4*, 357 (1959). – W. Klaus, Klin. Wschr. *42*, 1123 (1962).
[2] D. F. Wallach und T. L. Steck, Analyt. Biochem. *6*, 176 (1963). – D. F. Wallach, D. M. Surgenor, J. Soderberg und E. Delano, Analyt. Chem. *31*, 456 (1959). – S. Zepf, Zeiss-Mitteilungen, im Druck.