diesen Fasern und Zellen ein inniger Zusammenhang besteht (sekreto-motorischer Effekt?).

In der Pars distalis der Adenohypophyse fanden wir spezielle Nervenendigungen vor. Die innervierenden Fasern weisen hier nicht nur die beschriebenen keulen- bzw. ringförmigen Endigungen auf, sondern enden manchmal in einem, einige Drüsenzellen umgebenden, feinen Netzwerk (Figur 3 und 4). Ursprung und Wirkungsmechanismus dieser, spezielle Endigungsformen aufweisenden Fasern sind noch nicht hinreichend geklärt, obwohl ihr Verlauf zu der Annahme berechtigt, dass sie ebenfalls vom Tractus hypothalamo-hypophysealis stammen. Ob ihnen eine neurosekretorische Funktion zuzusprechen ist, soll im Laufe weiterer Untersuchungen entschieden werden.

Summary. We demonstrated, in the pituitary of the animals examined, fibres which emerge from the Tr. hypothalamo-hypophyseal system and enter partly into the Pars intermedia and partly *via* the Pars tubularis into the Pars distalis and take their course directly towards the glandular cells. In the Pars distalis the nerve fibres end around the glandular cells in a special pericellular net.

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Inhibition of Precipitin Formation in the Guinea-Pig with Cyclophosphamide

Evidence has been presented that cyclophosphamide, an alkylating agent, inhibits primary formation of anaphylactic antibody and induces a specific tolerance to anaphylactic sensitization in the guinea-pig[1,2]. In that work the crisp, but qualitative, mark of death-in-acute-anaphylaxis was the end point. In studies here reported, the ability of cyclophosphamide to inhibit the formation of precipitating antibody in the guinea-pig is evaluated using a quantitative method of double diffusion in gel for antibody determination[3]. Further, dosage schedules of antigen in saline solution as against similar amounts of antigen in complete Freund's adjuvant are compared and the antibody inhibiting abilities of two other cancer chemotherapeutic drugs, viz. vincaleukoblastine and 6-mercaptopurine, have been tested.

Methods. Antigen throughout was five times recrystallized egg albumin[4] prepared at a half-percent concentration in physiological saline. When adjuvant was used, equal parts of Freund's complete adjuvant[5] and egg-saline solution were emulsified immediately prior to injection. All antigen injections were intraperitoneal (IP). Animals were closed-colony randomly-bred Hartley strain, albino guinea-pigs, weighing approximately $^1/_2$ kg. Cyclophosphamide and vincaleukoblastine were prepared in physiological saline solution. 6-Mercaptopurine was suspended in cotton seed oil 150 mg/cm³. Blood was secured *via* intercardiac puncture under light ether anesthesia at 2, $4^1/_2$ and 8 weeks after first antigenic stimulation; and additionally, for the cyclophosphamide treated animals, at $2^1/_2$ weeks following second antigenic stimulation.

Antibody concentration was measured using the technique of PREER, which quantitates on the basis of the position of precipitation band in gel agar in capillary tubes[3].

Results. (1) *Saline cyclophosphamide:* Sensitizing schedule is shown in Figure 1. Serum from control animals showed good antibody concentration at 2 weeks (8/8), $4^1/_2$ weeks (7/7), and 8 weeks (5/5). The cyclophosphamide treated animals had no detectable antibody at 2 weeks (8/8) and 8 weeks (7/7); at $4^1/_2$ weeks 11 of 12 animals had no detectable antibody and the remaining guinea-pig had trace (but definite) antibody.

(2) *Failure of vincaleukoblastine and 6-mercaptopurine:* Sensitizing schedule is shown in Figure 1. Comparably timed and somewhat more toxic schedules of vincaleukoblastine and 6-mercaptopurine did not inhibit antibody formation; the antibody concentrations of these animals were indistinguishable from those of the control group. The comparative mortality of the several drugs is shown in the Table.

(3) *Immune tolerance induced by treatment with egg-cyclophosphamide:* A group of the cyclophosphamide treated animals that had been exposed to antigen $5^1/_2$ weeks previously and a new control group were injected with egg albumin (1 mg IP alternate days × 3). No additional cyclophosphamide was used. Blood was drawn $2^1/_2$

Comparative mortality
approximately $^1/_2$ kg guinea-pigs

	3 week survivors
Cyclophosphamide 10 mg/dx 14	16/20
Vincaleukoblastine 11 γ/dx 10	12/20
6-mercaptopurine 150 mg in oil day 1 and day 5	7/21

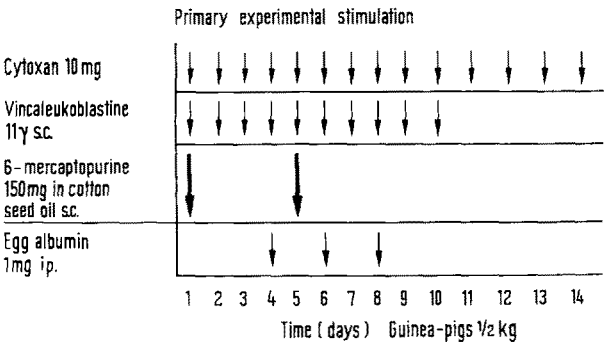

Fig. 1. Dose and time relationships of drugs and antigen are shown. Control animals received egg albumin and no drug. The three different experimental groups were given egg albumin and one of the 3 test drugs.

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[3] J. R. PREER JR., J. Immunol. *77*, 52 (1956).
[4] Pentex Company.
[5] Difco.

weeks later. Control animals showed good antibody concentration (8/8), whereas the 16 cyclophosphamide pretreated guinea-pigs divided into the following qualitative distinct groups: (a) no detectable antibody – 9; (b) trace antibody – 5; and (c) good antibody (overlaps with controls) – 2. As a group, the guinea-pigs previously treated with cyclophosphamide and egg albumin showed inhibition of formation of anti-egg precipitating antibody.

(4) *Stimulation of cyclophosphamide animals with antigen emulsified with Freund's complete adjuvant:* The cyclophosphamide-antigen schedule was identical with that of the initial experiment as outlined in Figure 1, except that antigen was emulsified in complete Freund's adjuvant. Blood was drawn at 3 and at 4 weeks after the first antigen injection. At 3 weeks the Freund egg albumin controls showed excellent titers whereas the experimental animals had good, but more than 4-fold weaker titers (Figure 2). However, at 4 weeks the titers in experimental and control Freund egg albumin animals were both excellent and indistinguishable. Under the conditions of the experiment cyclophosphamide had only temporarily and incompletely damped the antibody formation response to the strong antigenic stimulus of Freund's egg albumin sensitization.

Discussion. Cyclophosphamide is an alkylating agent that is used in the treatment of human malignancies, particularly those of the lymphoma group. In both man and guinea-pig appropriately high dosage schedules will regularly produce a profound leukopenia. Although the granulocytes are more sensitive than the lymphocytes, a 20 day course of cyclophosphamide 10 mg per day, when given to 500 g guinea-pigs, may depress the total peripheral leukocyte count to less than 200 cells/mm³. A high price in morbidity and mortality is paid for achieving such a profound leukopenia.

Specific inhibition of primary antibody formation has been demonstrated with X-rays and a number of antimetabolites and nitrogen mustard drugs in several animals[6-9]. Further, SCHWARTZ and DAMESHEK[10] have shown that the inhibition of primary antibody stimulation with 6-mercaptopurine in the rabbit may produce a long lasting refractoriness to further stimulation with given antigen (specific acquired immune tolerance). Our findings reported above demonstrate a parallel effect in the guinea-pig with cyclophosphamide. It is evident that the inhibition of primary antibody formation is more easily achieved than the production of immune tolerance.

It is interesting that the barrier to antibody formation can be well-nigh overcome *via* the use of Freund's adjuvant. This finding would be congruent with the report that 6-mercaptopurine failed to inhibit primary formation of circulating antibodies in rabbits sensitized with albumin emulsified in Freund's complete adjuvant[11]. It would also be a model for those experiments in which circulating antibody formation was delayed, but not prevented, by cancer chemotherapy drugs[12]. We have reason to feel that a comparable explanation would hold for the only transient inhibition of delayed hypersensitivity with cyclophosphamide and other drugs – the antigenic stimulus being too profound.

MAIBACH and MAGUIRE failed to achieve any significant inhibition in guinea-pigs treated with 6-mercaptopurine on a schedule comparable to that successfully used in rabbits by SCHWARTZ and DAMESHEK using lethal anaphylaxis as an end point[13]. Previous workers have had a similar experience[14]. The lack of success may represent a species' difference or, perhaps, a failure to develop optimum schedules of antigen and drug. It is interesting that the guinea-pig leukocyte (and lymphocyte) is quite sensitive to cyclophosphamide and relatively resistant to 6-mercaptopurine and vincaleukoblastine, in the schedules used.

Zusammenfassung. Cyclophosphamid (aber nicht 6-Mercaptopurin oder Vincaleukoblastin) verhindert die primäre Antikörperbildung beim Meerschweinchen. Auch bei fortgesetzter Stimulation mit dem spezifischen Antigen bleiben die Tiere relativ refraktär. Dieser Hemmeffekt wird weitgehend aufgehoben, wenn das Antigen mit Freund's Adjuvans injiziert wird.

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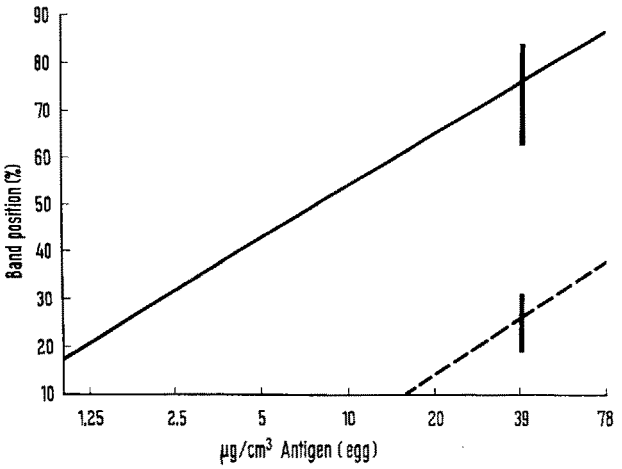

Fig. 2. —— Freund egg albumin stimulated animals at 3 and 4 weeks; Freund egg albumin + cyclophosphamide animals at 4 weeks. ----- Freund egg albumin + cyclophosphamide animals at 3 weeks. Coincident cyclophosphamide treatment delayed appearance of maximum titers of precipitating antibody. The range within each group is indicated by the vertical bars.

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