Results. Test results in the Normotest assay for serially diluted individual and pooled blood samples, obtained by aortic puncture in normal rats, were used to construct a reference curve by plotting the clotting time against calculated coagulation factor activity (Figure 1). The results of the individual and the pooled samples fit the same hyperbolic curve.

The Normotest coagulation times in blood samples taken from the tail of rats on anticoagulant therapy were plotted against the mean plasma level of factors II, VII, and X assayed in a simultaneously collected sample. The reference curve for the initial stage of anticoagulation, using a warfarin dose completely blocking[9] the clotting factor synthesis, can be seen in Figure 2; it is identical in shape with the saline curve until very low levels of coagulation factors are reached and relatively too short Normotest times are found.

The data obtained in long-term anticoagulation, from 4 to 21 days, yield a curve that deviates from the other curves, especially in the area below 20% coagulation factor activity, in the sense that Normotest times are relatively too long (Figure 2).

During the long-term treatment, from the 12th day on, succesively 14 of the 21 rats showed a drop in hematocrit and died from bleeding (confirmed on autopsy).

Discussion. A reagent for the control of anticoagulant therapy should be sensitive for the depression of clotting factors of the species treated. For the control in rats, we found human brain and ox-brain thromboplastin (Thrombotest) unsuitable because of the flatness of the standard reference curve. Rat-brain thromboplastin, laborious to produce, and rabbit-brain thromboplastin (Normotest) appeared to be sufficiently sensitive. The only modification in the Normotest assay procedure was the use of 20 µl blood as compared to 10 µl used for human blood. With 10 µl of rat blood in the lower clotting factor activity levels were too long and irreproducible clotting times were found.

The saline dilution curve assayed in Normotest, a reagent not sensitive to changes in factor V and fibrinogen, should be identical to a curve constructed with test-results in anticoagulated rats, which is indeed the case for the curve constructed with data from the initial phase of anticoagulation. Below the values of 10% coagulation factor activity, relatively too short Normotest times are found, which can be explained by the fact that these rats were bled 5 times within 24 h which caused a drop in hemotocrit value, amounting to about 4%, giving relatively more plasma in the samples[16].

An essential deviation from both the saline curve and the curve based on the initial stage of anticoagulation is observed for the curve based on long-term anticoagulant samples, especially below the 20% coagulation factor level, which we believe to be caused by inhibition of the Normotest assay by PIVKAs.

It could be argued that in long-term anticoagulation all 3 factors show equally depressed activity, whereas in the initial stage factor VII (fastest turnover rate[9]) activity will decrease most rapidly. Even when we excluded factor VII results in the calculations, we found the same discrepancy between the 2 curves.

It might be that PIVKAs in the rat appear in a later stage of anticoagulation as compared to humans[17], because also SUTTIE[18], using immunochemical methods, could not demonstrate abnormal prothrombin (PIVKA-II) during the initial stage of anticoagulation in rat blood.

For practical purposes, e.g. the study of interactions between other drugs and oral anticoagulants, rats should not be anticoagulated below 15% activity of the coagulation factors of the prothrombin complex, which corresponds to 145 sec Normotest, because of the high bleeding incidence. Furthermore it should be realized that Normotest times in the initial stage of anticoagulation differ so much in significance from those during long-term treatment that the 2 different curves have to be used[19].

Résumé. La méthode d'analyse à l'aide du réactif Normotest du contrôle en laboratoire de l'anticoagulation par les dérivés des cumarines montre chez les rats une sensibilité propre pour la diminution de l'activité des facteurs de coagulation et aussi pour l'action inhibitrice des PIVKA, lesquels se manifestent dans le sang avec un certain délai. Un traitement de longue durée à moins du 15% en moyenne de l'activité des facteurs de coagulation est déconseillé à cause des très grands risques d'hémorragie.

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Effect of Cytosine Arabinoside on Thyroid Autoimmunity in Guinea-Pigs

Cytosine arabinoside (Ara-C), a pyrimidine nucleoside analog, selectively interfers with DNA synthesis at the S-phase of the cell cycle[1]. The primary effect of this antimetabolite in all mammalian species is exerted on the bone marrow and other rapidly dividing cell populations[2]. Regarding the immune response, Ara-C has been found to have a marked effect on the rapidly dividing lymphoid cells during the log phase of humoral antibody production[3]. In thyroid antoimmune systems, antimetabolites have been shown to be uniquely qualified as agents to probe the various phase of the autiommune response[4]. Ara-C has been successfully employed as an immunosuppressant in allergic encephalomyelitis but is untested concerning autoimmune thyroiditis[5]. In view of these findings, the present study was designed to investigate the effects of Ara-C on thyroid autoimmune induction by i.p. ad-

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Table I. Anti-thyroid guinea-pig antiserum was titered using passive hemagglutination of sensitized sheep red cells in micro-Takatsky plates

Time of bleeding	Group No.[a]	Responsive (%)	Range of titer	Average titer[b]
18 Days	II	6/12 (50%)	1:20–1:1280	1:180
	IV	5/12 (42%)	1:80–1:1280	1:210
36 Days	II	4/12 (33%)	1:192–1:1280	1:132
	IV	2/8[c] (25%)	1:40–1:320	1:100

[a] See text for explanation of group member. [b] Average titer was determined by log transformation since titer data is normally a skewed distribution. [c] Four of this group died.

ministration of Ara-C concurrent with and following antigen immunization.

The present study utilized 48 male, Hartley guinea-pigs divided into 4 groups comprizing 12 animals per group. Group I was treated with a 50:50 mixture of saline and Freund's complete adjuvant; Group II with a similar mixture of homologous thyroid extract and adjuvant; Group III with saline-adjuvant and Ara-C; and Group IV with thyroid-adjuvant and Ara-C. The guinea-pigs were skin tested between day 30 and 34 of the experiment. All animals were bled on days 0, 18 and 36 of the study. At the final bleeding (day 36) all the guinea-pigs were autopsied. Ara-C (U-19, lot No. 9570-BDA-18) was injected i.p. twice daily (10 mg/kg body wt.), 3 times a week, for 5 weeks. The paired thyroid glands from 20 to 40 guinea-pigs were employed to prepare a saline organ extract following the method of WITEBSKY and ROSE[6].

Autoimmune thyroiditis was induced in the guinea-pigs by methods forwarded by FLAX[7] and MIZEJEWSKI et al.[8]. Anti-thyroid antibodies were titrated by the passive hemagglutination (PHA) of sheep red cells tanned by the method of BUTT[9]. Skin tests were performed by previously published methods[10]. All animals were autopsied 36 days following the first antigen injection and the thyroid, liver, spleen, lymph nodes, bone marrow, and thymus were removed at autopsy. Tissue sections were stained with hemytoxalin and eosin. The grading of thyroiditis histopathological lesions has previously been described[11] and has been modified for use with the guinea-pig. At the time of each animal's bleeding mentioned above, the blood samples were subjected to reticulocyte and white blood cell counts and microhematocrit evaluation. In addition, measurements of total body weight were recorded.

The leukocyte and reticulocyte counts were drastically depressed in the animals treated with Ara-C as compared to the non-treated animals. Results of the microhematocrit assay showed a slow, continuous depletion of blood cells throughout the experiment. With reference to body weight, the drug-treated animals displayed a reduction when compared to the non-treated animals. In summary, the effect of Ara-C injection into guinea-pigs was an overall depression in hematopoesis and a loss in body weight.

The PHA antibody titrations using red cells sensitized with thyroid antigen are presented in Table I. Antibody titers appeared generally in 40–50% of the immunized animals. At day 18, the range of the antibody titers (1:20 to 1:1280) and the mean titers for Group II and IV were essentially the same. Slightly more animals (50%) in Group II responded to antigenic stimulation than did Group IV (42%); however, this difference was not significant. By day 36, the present response has declined to 33% and 25% in Groups II and IV, respectively. The percentage drops in Group II (the non-drug treated group) from 50% to 33% probably reflect the normal decline kinetics of the humoral antibody response. It is noteworthy that the corresponding percentages were the same in Group IV, but the drop in the average titer was greater. However, the averages titers between Group II and IV at 18 and 36 days, respectively, were not significantly different.

The percent of animals responding to the skin tests in Groups II and IV was 58 and 75%, respectively. Animals in Group I had not been originally immunized and responded in a non-specific fashion displaying reactions (0.2–0.6 cm) considerably smaller than the other groups. A greater mean diameter was observed in Group IV (4.8 cm) as compared to Group II (3.6 cm). It was noted that the range of intensity of Group IV was greater than in Group II.

The use of Ara-C did not abate the presence of thyroid histologic lesions as seen in Table II. Both Groups II and IV included animals displaying histopathologic lesions of grades one and two. However, fewer animals (25%) in Group IV displayed grade-two lesions than did comparable animals in Group II (42%). Both groups, however, had similar percentages of animals with thyroid histologic damage (83% and 75%). Although the presence of Ara-C did not abolish damage to the thyroid gland, it may have reduced the severity of the histologic lesions involved. Many of the guinea-pigs displayed focal non-

Table II. The severity of the thyroid histopathological lesions is presented for the Guinea-Pigs immunized with thyroid gland antigen

Group No.[a]	Animals with lesions (%)	Assigned thyroiditis grading			
		0	+1	+2	Total
II	10/12 (83%)	2 (16%)	5 (42%)	5 (42%)	12
IV	6/8[b] (75%)	2 (25%)	4 (50%)	2 (25%)	8

[a] See text for explanation of group numbers. [b] Four of this group died.

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specific areas of inflammation in the lungs which were characterized by the presence of nodular mononuclear cell infiltrates. Secondary to the lungs, other inflammatory observations were vacular (lipidic) changes associated with small areas of necrosis and inflammation, and small aggregates of polymorphonuclear leucocytes in the liver. Finally, the bone marrow was acellular and, occasionally, hyperplastic lymph nodes were noted.

In the present study, the use of Ara-C produced a varied response regarding the 3 aspects of the autoimmune response, namely, 1. humoral anti-body production; 2. delayed hypersensitivity; and 3. immunopathological lesions. The drug appeared to cause a slight depression in the humoral antibody response to homologous thyroid antigen by day 36 postimmunization. The skin test response indicated a more intense delayed hypersenstive response in the drugtreated, immunized animals than in comparable controls. Also, more of the guinea-pigs treated with Ara-C responded to skin testing. In this regard, one cannot rule out the possibility that non-immune, inflammatory changes had influenced the skin response. Immunopathological lesions, the third facet of the autoimmune response, was less influenced by the presence of Ara-C; nevertheless, fewer severe lesions were observed in the drug-treated animals. In summary, Ara-C produced only a mild suppressive effect on thyroid autoimmune induction and was unsuccessful in preventing tissue damage.

The cellular immune aspects of the autoimmune response appeared independent of the humoral response for the production of thyroid lesions. Once the cellular response had been initiated for action, it was not susceptible to the continuous insult and exposure to the body caused by the presence of Ara-C. The antigen sensitive dividing cell populations were either sheltered from Ara-C exposure or had undergo sufficient cell division before the drug could be effective. Thyroglobulin or thyroid antigen is thought to require processing by thymic derived cells or macrophages before presentation to the antibody-receptors of sentivive cells[12]. This step may not have been affected by the drug since the cellular immune responses were not greatly influenced and thyroid histologic lesions were evident. In addition, histological changes were not prevalent in either the thymus, spleen, or lymph nodes. Thus, the adminsitration of cytarabine via the i.p. route during immunization does not appear to inhibit the cellular immune response in thyroiditis.

Zusammenfassung. An einer Untersuchungsserie experimentell induzierter Autoimmunthyreoiditis beim Meerschweinchen wird gezeigt, dass Cytosine Atabinoside nur einen geringen suppressiven Effekt auf die Autoimmuninduktion hat und die Gewebezerstörung nicht zu verhindern vermag.

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The Effect of Prostaglandin on Tumour Implantation

The prostaglandins (PGs) are ubiquitous tissue hormones with a wide variety of biological activities[1]. They mediate i.a. the inflammatory and allergic response[2,3] by promoting the diapedesis[4] and migration[5] of leucocytes.

The rejection of a non-malignant homograft is the result of a local delayed hypersensitivity reaction mounted by the recipient[6]. Malignant homografts fail to elicit this response[7]. It is believed that tumour secretes a PG antagonist and that the PG deficiency so induced is the cause of the immunological paralysis in the vicinity of tumour[7,8].

On this hypothesis, the addition of large amounts of PG to the tumour inoculum would counterbalance the PG deficiency, re-instate the delayed hypersensitivity response and so lead to the rejection of the graft. The present communication reports on some relevant experiments.

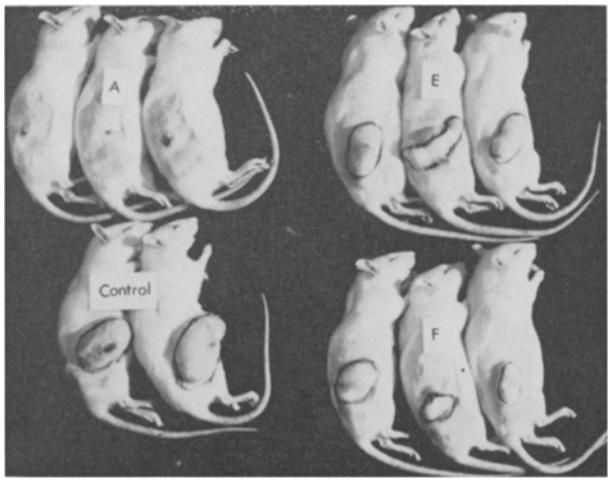

Fig. 1. Tumour growth in 4 groups of rats 3 weeks after inoculation of tumour suspended in solutions of PG A, E and F or solvent. The recipients of PGA have small ulcers but no tumour.

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Tumour growth in rats inoculated with tumour suspended in solutions of 1 mg/ml PG A2, E2 and F2 or solvent

Site of inoculation	PGA2	PGE2	PGF2	Solvent
Subcutaneous weanling	16/31	6/6	6/8	27/27
Intramuscular weanling	16/16	1/1	2/2	15/15
Subcutaneous neonate	4/4	4/4	—	10/10