anti-A production, served as negative controls. A massive transfusion, 200 ml whole blood, was attempted in 2 dogs: 1 pre-treated and 1 non-pretreated for anti-anti-A production.

Results. Hemolytic anti-A antibodies were produced in all 4 rabbits (1:32). After isolation, the pure anti-A antibody's hemolytic activity was 50% that of the rabbit anti-A serum (1:16). Canine anti-anti-A serum precipitated both rabbit anti-A serum and the isolated anti-A antibodies. All canine anti-anti-A serums precipitated with either rabbit anti-A serum or isolated anti-A antibodies produced no hemolysis when mixed with type A blood cell suspension. The hemolytic activity of rabbit anti-A serum or isolated anti-A antibodies was not impaired when normal canine serum was substituted for the canine anti-anti-A serum, indicating that the hemolytic activity was not impaired by normal serum components. After 12 transfusions, sera of 2 of 3 dogs pre-treated for anti-anti-A production lacked the ability to hemolyse type A erythrocytes, while sera of both the untreated dogs produced hemolysis. The dog not pre-treated for anti-anti-A production died of hemolytic reaction during the transfusion. The pre-treated dog survived the massive transfusion and exhibited no side effects, hemoglobinemia or hemoglobinuria. He is still alive and well.

Discussion. Anti-antibody was produced and its action demonstrated using the immunological triangle concept. The results achieved suggest that anti-anti-A antibody neutralizes anti-A antibody in vivo and prevents a demonstrable titer in hemolytic titration tests in vitro. The neutralization of anti-A antibody by anti-anti-A antibody has been shown to prevent the transfusion reaction in recipients pre-treated for anti-anti-A antibody production[9].

Zusammenfassung. Durch ein immunologisches Dreieck-System (Hund-Kaninchen-Hund) wurde ein Anti-Anti-körper bei Hunden erzeugt und seine Wirkung in vitro und in vivo demonstriert. Die Anti-Antikörper-Methode bietet neue Möglichkeiten, dem Problem der Transplantat-Abstossung und der Therapie der Krankheiten immunologischen Ursprungs näherzukommen.

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[9] This investigation was supported by Public Health Service grant No. CA 02624 and the Detroit General Hospital Research Corp. and Metropolitan Hospital Research grant No. 208-12.

Immunosuppressive and Specific Antimitotic Effects of Ovalicin

In a previous report[1] the immunosuppressive effect of ovalicin ($C_{16}H_{24}O_5$, isolated from cultures of *Pseudeurotium ovalis*[2]) in rodents was described: marked reduction of antibody production and of the number of antibody-forming cells in mice after stimulation with sheep erythrocytes and inhibition of the symptoms of experimental allergic encephalomyelitis in rats. Leukopenia was not observed in these experimental animals. It is the purpose of the present report to describe the effect of a single dose of ovalicin on primary and secondary hemagglutinin response in mice and on survival of skin grafts between mouse strains with identical H-2 locus. We have also investigated the effect of the compound on splenic weight in mice injected with red blood cells and/or with cells of L-1210 leukemia; furthermore, the frequency of mitoses after administration of ovalicin was evaluated in sections of spleen and jejunum of immunized and non-immunized animals.

Materials and methods. (Albino × DBA/2)F_1, BALB/c and DBA/2 male mice, 2–3 months old, were obtained from our own animal farm. 0.5 ml of a 10% suspension of washed sheep red blood cells (SRBC) in saline was administered i.v. for immunization. Hemagglutinin titres in sera were determined for each individual animal by serial 2-fold dilutions with Takatsy's microtechnic. L-1210 mouse leukemia cells were obtained from ascitic growth in (albino × DBA/2)F_1 mice; 10^6 cells were injected into the hind leg of experimental animals. Fitted 'pinch' grafts of skin from male DBA/2 donors were transplanted to male BALB/c recipients according to the method of Billingham and Medawar[3]. The survival time of the grafts was determined by daily inspection, the criterion being the endpoint of epithelial survival. For evaluation of the mitotic index in spleen and jejunum the organs were fixed in Bouin's fixative and stained with hematoxylin and eosin; the number of mitoses was then counted by a person who did not know from which animal the sections came. For each spleen, the mitoses in 150 fields, and for the jejunum mitoses/1000 epithelial cells in the crypts were counted (magnification × 1000). Ovalicin was administered as a suspension in 0.5% carboxymethylcellulose in saline either orally or i.p. The treatment schedules for each experiment are given with the results.

$$\text{S.D.} = \text{standard deviation} = s = \sqrt{\frac{\Sigma (x - x_i)^2}{n-1}}$$

Results. The primary hemagglutinin response in mice, as determined on day 7 or 14 after immunization with SRBC, was strongly inhibited by a single dose of ovalicin. Partial tolerance to the standard secondary challenge with SRBC was found when the primary response was inhibited with an oral dosage of 1200 mg/kg of ovalicin. When animals were treated with the drug on the day of the second challenge with SRBC only, there was but a slight inhibition of the response (Table I).

Table II shows the effect on skin graft survival in mice of a single dose of ovalicin, injected i.p. on different days

[1] S. Lazáry and H. Stähelin, *The Immune Response and its Suppression*, (Symposium of the Swiss Society of Allergy and Immunology, Davos, March 25–28, 1968), in press.
[2] H. P. Sigg and H. P. Weber, Helv. chim. Acta *51*, 1395 (1968).
[3] R. E. Billingham and P. B. Medawar, J. exp. Biol. *28*, 385 (1951).

after transplantation. The best effect was obtained with drug treatment 3–4 days after grafting. None of the animals died during the experiment.

It is known that after i.v. SRBC stimulation, there is, in the early stage of the specific response, an increase of spleen weight; this in an expression of rapid division of antibody-forming cells. The Figure shows that high doses of ovalicin reduce the spleen weight in immunized animals to a level which is close to that of non-immunized mice. Similarly, spleen weight increase due to infiltration and multiplication of leukemia L-1210 cells is repressed by ovalicin, in a hybrid host (Figure) as well as in the isologous host (Table III). Despite this inhibition of spleen weight increase, ovalicin does not prolong substantially the survival time of mice inoculated s.c. with L-1210 cells[4]. The greatest inhibition of spleen weight increase with ovalicin was found in mice which had been inoculated with L-1210 cells and in addition stimulated with SRBC (Figure).

Since increase in spleen weight after antigenic stimulation is due to mitotic activity of cells in the spleen[5], an attempt was made to determine the influence of ovalicin on the mitotic index in spleens of mice before and after antigenic stimulation; the number of mitoses in the jejunal crypts of the same animals was also counted. From Table IV it can be seen that rather high doses of ovalicin are required to depress significantly the mitotic index in the spleens of non-stimulated mice, while in immunized mice lower doses are quite active. The specificity of the antimitotic effect of ovalicin for immunologically stimulated cells and for tumour cells (L-1210) in the spleen

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Table I. Inhibition of primary and secondary hemagglutinin response in (albino × DBA/2)F_1 male mice with single treatment of ovalicin

Day 0	Day 7	p	Day 14	p
–	1.4 ± 0.9[a]			
SRBC[b]	9.9 ± 0.9			
SRBC + 600[c]	5.9 ± 1.5	< 0.0005		
SRBC + 1200	1.5 ± 0.5	< 0.0005		
SRBC			10.6 ± 1.1[a]	
SRBC + 600			6.8 ± 1.6	< 0.0005
SRBC + 1200			3.6 ± 1.1	< 0.0005
SRBC	SRBC		10.7 ± 1.3	
SRBC + 600	SRBC		9.8 ± 1.4	< 0.05
SRBC + 1200	SRBC		6.3 ± 1.4	< 0.0005
SRBC	SRBC + 600		9.9 ± 1.6	> 0.05
SRBC	SRBC + 1200		8.6 ± 0.9	< 0.0005

12 animals/group. All animals survived until sacrifice, 7 or 14 days after drug application. [a] Hemagglutinin titre (expressed as power of 2), [b] sheep red blood cells, [c] mg/kg ovalicin orally.

Table II. Effect of ovalicin on survival of DBA/2 skin grafts in BALB/c mice

Drug treatment after transplantation on day...	Graft survival (days)	p
Controls	10.0 ± 1.4	
2	13.8 ± 2.9	< 0.025
3	16.1 ± 4.1	< 0.01
4	15.0 ± 1.2	< 0.0005
5	13.8 ± 0.4	< 0.0005

Single treatment of 300 mg/kg i.p. on different days after transplantation. 5 animals/group.

Table III. Effect of ovalicin on the spleen weight in DBA/2 mice inoculated with L-1210 cells

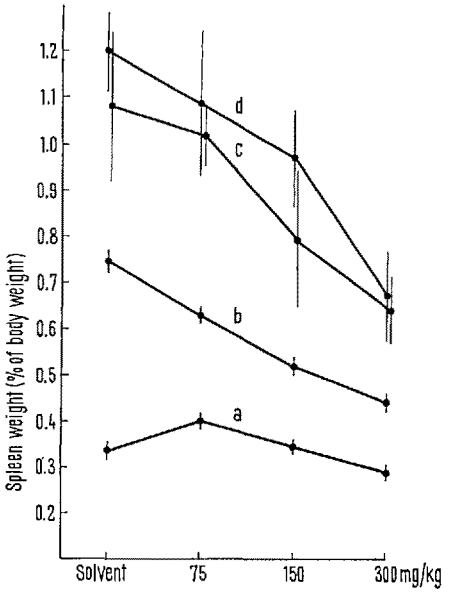
L-1210 cells day 0	Treatment (p.o.) from day 0 to 7	No. of animals	Spleen weight[a] on day 8	p
No	None	5	0.39 ± 0.04	
Yes	Solvent	8	1.36 ± 0.31	
Yes	15 mg/kg	4	0.80 ± 0.37	< 0.0125
Yes	30 mg/kg	5	0.99 ± 0.30	< 0.05
Yes	60 mg/kg	5	0.74 ± 0.11	< 0.0025
Yes	120 mg/kg	5	0.46 ± 0.06	< 0.0005

[a] % of body weight.

Table IV. Effect of ovalicin on frequency of mitoses in spleen and jejunum of immunized and non-immunized mice

Treatment	Spleen mitoses/field	p	Jejunum mitoses/1000 cells	p
1 none	0.25 ± 0.03		20.8 ± 2.6	
2 solvent	0.33 ± 0.11		19.0 ± 2.2	
3 75 mg/kg	0.31 ± 0.09	> 0.05 (2)	19.2 ± 2.6	> 0.05 (2)
4 150 mg/kg	0.34 ± 0.11	> 0.05 (2)	20.0 ± 2.2	> 0.05 (2)
5 300 mg/kg	0.16 ± 0.06	< 0.025 (2)	19.8 ± 1.7	> 0.05 (2)
6 SRBC	0.87 ± 0.11		20.0 ± 3.4	
7 SRBC + solvent	0.95 ± 0.13		23.0 ± 5.4	
8 SRBC + 75 mg/kg	0.51 ± 0.11	< 0.0025 (7)	21.3 ± 5.0	> 0.05 (7)
9 SRBC + 150 mg/kg	0.42 ± 0.05	< 0.0005 (7)	20.5 ± 6.2	> 0.05 (7)
10 SRBC + 300 mg/kg	0.39 ± 0.15	< 0.0025 (7)	20.0 ± 3.4	> 0.05 (7)

Animals treated as in curve a and b of the Figure. Figures in parentheses after p-value indicate line with which comparison is made in Student t-test. 4 animals in each group.



Effect of ovalicin on spleen weight in (albino × DBA/2)F_1 male mice. The spleens were weighed on day 7, 4 h after the last drug application. Ovalicin was given i.p. to all 4 groups on days 5, 6 and 7. Curve a, drug only. Curve b, SRBC i.v. on day 4. Curve c, 10^6 L-1210 cells on day 0. Curve d, L-1210 cells on day 0 and SRBC on day 4. Each dot represents the mean ± S.E.M. of 4–6 animals.

becomes also evident from comparison of the mitotic index in the spleen with that in the jejunum (Table IV) and from the Figure; similarly, there seems to be no inhibition of proliferation in the hematopoietic system, since rats[1] and rhesus monkeys[6] showed normal leucocyte values after treatment with immunosuppressive doses of ovalicin.

Zusammenfassung. Ovalicin, isoliert aus dem Kulturfiltrat von *Pseudeurotium ovalis*, hemmt die Bildung von Antikörpern schon nach einmaliger Applikation. Es kommt zur Ausbildung einer partiellen immunologischen Toleranz. Die Abstossungszeit von homologen Hauttransplantaten wird bei Mäusen durch eine einmalige Ovalicinbehandlung signifikant verlängert. Die Substanz hemmt den Anstieg des Milzgewichtes bei Mäusen, die mit Schaferythrozyten immunisiert und/oder mit lebenden Leukämie-L-1210-Zellen geimpft wurden; letzteres gilt auch für den isologen Wirt (L-1210-Zellen in DBA/2-Mäusen). Die Hemmung des Milzgewichtanstieges durch Ovalicin geht parallel mit einer Reduktion des Mitoseindex in der Milz von immunologisch stimulierten Tieren; die Mitosenzahl im Darmepithel wird hingegen nicht beeinflusst.

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Biological and Medical Research Division, Sandoz Ltd., Basel (Switzerland), 30 August 1968.

[6] S. Lazáry, unpublished results.

Hypodiploidy of Bone Marrow in Hypertransfused Mice Stimulated with Erythropoietin

The karyological examination of the bone marrow of persons carrying body burdens of ^{90}Sr and ^{226}Ra revealed, apart from other abnormalities, hypodiploidy of marrow elements[1]. However, the question of the mechanism by which the ionizing radiation induces the decrease of ploidy still remains unexplained. In this connection, attention was paid to some earlier findings[2,3] which gave suggestive evidence of the fact that the loss of chromosomes takes place in the course of maturation of erythroid precursors. Providing this assumption be confirmed, varying representation of haemopoietic series and maturation stages in the bone marrow could participate in the change of ploidy after irradiation.

The model of the bone marrow with varying proportions of individual generations of red precursors was prepared by using hypertransfused female C 57 Bl mice in which the bone marrow was examined at the intervals of 24, 48 and 72 h after the administration of erythropoietin. The mice were injected i.p. on 2 successive days with 1 ml of 80% suspension of isogeneic donor red cells. It was assumed that on the sixth day after the first transfusion the bone marrow had been cleared from differentiated erythroid precursors.[4,5] At that time erythropoietin was administered in the amount of 16 U in one experiment and of 6 U in the other, both levels representing the submaximum stimulation, however. Intact animals and hypertransfused ones not treated with erythropoietin were investigated as well. At the time of sacrifice haematocrit was checked and in all animals the values were well above the critical level,[6,7] being at least 60%.

The mice were injected i.p. 2 h prior to sacrifice with colchicine (1×10^{-5} g/g body weight) and the bone marrow obtained from the femur was immediately treated cytologically. After hypotonic treatment and fixation, the smears were prepared, dried over the spirit flame and stained with Giemsa. Chromosomes in 100 metaphases from each animal were counted by drawing from microfilm. The metaphases with non-reproducible count and/or without distinct cytoplasmatic area were discarded. The cells with chromosomal counts $2n = 40$ were classified as euploid. The chromosomal count in our study ranged from 5–47. In Figure 1 the hypodiploid and euploid fractions are included. The first pair of columns shows the situation in the intact controls. The second pair of columns depicts the decrease of hypodiploidy in hypertransfused mice. The next 3 pairs of columns represent the values obtained at

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