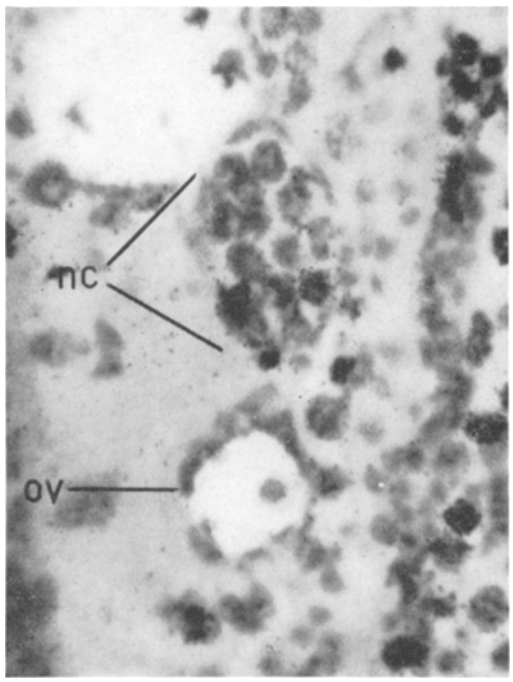

Fig. 2. Parthenogenetic ovary showing thymidine H^3 incorporation in diploid nuclei of nurse cells. Stained by Feulgen. × 980. n.c. = nurse cells; ov = ovocyte.

Diploid nurse cells are functioning continuously, since in a parthenogenetic ovary a great many ovocytes reach maturity one after the other, passing through all stages of development to produce the embryos. The nurse cells always retain, in such cases, their characteristic appearance right from the beginning of their differentiation[3] and therefore thymidine H^3 incorporation cannot be ascribed to continuous endomitotic divisions. It can therefore be assumed that the active synthesis which occurs in the nuclei does not concern genetically stable DNA but a metabolic DNA. The above results thus add new weight to the assumption by former authors[2,4] that metabolic DNA may be synthesized in the nurse cells of amphigonic insects as well.

Riassunto. Nell'afide *Megoura viciae* le cellule nutrici diploidi dell'ovario partenogenetico e quelle poliploidi dell'ovario anfigonico si comportano in maniera analoga incorporando timidina H^3 durante l'accrescimento ovocitario. Tale incorporazione viene attribuita alla sintesi di DNA metabolico.

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Immunofluorescent Antibody Studies of a Murine Leukemia Virus: Comparison of Human, Bovine and Murine Systems

The immunofluorescent antibody test is specific and allows a visualization of the viral antigenic site in the S-63 murine leukemia system[1,2]. Sera from mice convalescing from S-63 virus infection react with the S-63 virus but fail to react with normal mouse antigens, presumably because of their viral antibody contents. The present study explores the question whether the S-63 leukemia antibodies react with human and bovine leukemia antigens.

Material and methods. The following materials were used as antigens in our study: normal and leukemic tissues of man, normal and lymphomatous bovine tissues[3], normal mouse, S-63 and GC virus-induced leukemic mouse tissues. In each case spleen, lymph node, liver, brain, and kidney were studied.

Of the types of leukemia studied, 2 were acute lymphoblastic; 6 acute myeloblastic; 1 acute myeloblastic (possibly erythroleukemia); 2 chronic lymphocytic; 1 chronic granulocytic; 4 lymphomas; 2 lymphoblastic lymphomas; 2 mast cell disease.

Cell suspensions and touch preparations were made from each, as previously described[1].

Antisera. Pools of sera were collected from: normal ICR mice (NM), convalescent S-63 infected ICR mice (LM), rabbits made immune tolerant to S-63 virus (ITM63L), normal rabbits (NR), rabbits made immune tolerant to normal human tissues and then challenged with human leukemic tissues (ITHL), normal human (NH), leukemic human (LH), human volunteers injected with leukemic antigens (HV)[4], and laboratory workers handling leukemic antigens (LW)[5].

Immunofluorescence techniques. The direct, indirect, and indirect complement-fixing fluorescent tests of Cherry et al.[6] were employed. In all cases fluorescein isothiocyanate (FITC) was the fluorescing dye. Commercially prepared (Pentex Corp.) anti-guinea-pig serum conjugates were used in the indirect complement-fixing tests. All sera were absorbed against normal tissue antigens and silk fibroin[1].

All sera were tested by complement-fixation (CF), immunodiffusion (ID), and passive cutaneous anaphylaxis (PCA) tests. It is necessary to predetermine the presence of complement-fixing antibody. This is to insure a reliable indirect complement-fixing fluorescent test.

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Results. The results of the CF, ID, and PCA tests are shown in the Table. The human leukemic antigens were positive by CF tests when tested against antileukemic sera. Consistently positive reactions between convalescent S-63 mouse sera and human or bovine antigens could not be demonstrated by either the ID or PCA tests.

Immunofluorescence studies showed a cross-reaction between the mouse leukemia antibody and human leukemic antigen. The degree of fluorescence, whether employing the direct, indirect, or CF fluorescence tests, was never so strong in the heterologous as in the homologous system. Not all leukemic tissues fluoresced with the same intensity. Tissues from a patient with erythro-leukemia gave the greatest degree of fluorescence. In the mouse and human leukemic systems, fluorescence was observed in the cytoplasmic and interstitial spaces except in the case of erythro-leukemia. In that case and in the bovine lymphoma tissues both cytoplasm and nuclei fluoresced. Cross-reactions took place when convalescent S-63 mouse sera, immune tolerant anti-S-63 rabbit sera, immune tolerant anti-GC rabbit sera, immune tolerant anti-human leukemic rabbit sera, and anti-human leukemia human sera (from volunteers) were tested by immunofluorescent and CF tests against the bovine leukemic antigens. Control studies, using the same sera tested against normal bovine cells, failed to show fluorescence.

Discussion. The purpose of the present study was to compare the antigenic behavior of human, bovine, and mouse lymphomas. 20 cases of human leukemias and lymphomas, 2 bovine lymphomas, and the mouse S-63 and GC lymphoma were studied. On the basis of previous experience[1,2], a close antigenic relation was expected between human and mouse leukemias, and possibly bovine lymphomas[7].

In 1963, we reported[4] that specific antigens could be demonstrated in human leukemias by immunofluorescent and immunodiffusion techniques. The evolution of the S-63 and GC murine viruses in our laboratory[8,9] allowed a systematic analysis of the leukemic antigens. Antibodies obtained from mice that recovered from the S-63 virus infection reacted with both leukemic human and leukemic murine antigens[7]. Those antibodies also protected mice against infection with either the S-63 or the GC viruses[8]. Immunofluorescent and electron microscopic studies disclosed that the mouse leukemia inducing agent (antigen) was located in the cytoplasm of the cells or in the intercellular spaces, and that the agent was a virus[1,2]. It was thought that fluorescent dye tagged convalescent S-63 leukemic mouse sera might show that common antigens existed not only between the murine and human systems but also between the bovine system, and might also localize the antigens in the leukemic cells.

Using the RAUSCHER murine leukemia system, FINK et al.[10–12] have confirmed that immunofluorescent studies show antigens common to murine and human leukemias.

The use of convalescent sera obtained from S-63 virus-inoculated mice overcomes the objections of other systems in which non-specific cell antibodies and formalin treatment of antigens complicate the problem. Based on experience with the mouse system, it was anticipated that the cytoplasm and intercellular spaces of the infected cells would have fluorescence[1,7]. This was indeed observed in 19 of the 20 cases of human leukemias. A reaction was not demonstrable between antileukemic sera and normal tissues, or between normal sera and leukemic tissues. The specificity of the fluorescent antibody tests paralleled the positivity of the complement-fixation test. Sera reacting positively in the CF test yielded fluorescence when either conjugated or used in the indirect complement immunofluorescent test.

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In vitro testing of leukemic antigens

Antigens:	Controls			Human			Human			Bovine			Mouse					
	NH, NM, NB			AL, AM, Ly, MCD, CGL, AM (E)			CL			BL			LGCM			LS63M		
Test:	PCA	ID	CF	PCA	ID	CF	PCA	ID	CF	PCA	ID	CF	PCA	ID	CF	PCA	ID	CF
Sera																		
NM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LM	–	–	–	–	–	+	–	–	+	–	–	+	+	+	+	+	+	+
NR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ITHL	–	–	–	–	–	+	–	+	+	–	–	+	+	+	+	+	+	+
ITM63L	–	–	–	–	–	+	–	–	+	–	–	+	+	+	+	+	+	+
NH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HV	–	–	–	–	–	+	–	–	+	–	–	+	–	–	+	–	–	+
LW	–	–	–	–	–	+	–	–	+	–	–	+	–	–	+	–	–	+

Key to Table – *Tests:* PCA = passive cutaneous anaphylaxis; ID = immunodiffusion; CF = complement fixation. *Antigens:* NH = normal human; NM = normal mouse; NB = normal bovine; AL = acute lymphoblastic; AM = acute myeloblastic; Ly = lymphoma; MCD = mast cell disease; CGL = chronic granulocytic leukemia; AM(E) = acute myeloblastic (possibly erythroleukemia); CL = chronic lymphocytic; BL = bovine lymphoma; LGCM = leukemic GC virus infected mice; LS63M = leukemic S-63 virus infected mice. *Sera:* NM = normal mouse; LM = sera from mice recovering from S-63 virus infection; NR = normal rabbit; ITHL = immune tolerant rabbit anti-human leukemia; ITM63L = immune tolerant rabbit anti-S-63 virus; NH = normal human; LH = leukemic patients; HV = human volunteers challenged with leukemic antigens; LW = laboratory workers.

Different tissues gave different degrees of fluorescence and homologous systems usually gave a greater degree of fluorescence than interspecies reactions. This might be explained on the basis of slight differences in antigenic relation, such as those within various virus strains when cultivated in different mammalian tissues; or, might be attributed to differences in the viruses, notwithstanding antigenic similarities.

Zusammenfassung. Antikörper bei genesenden Mäusen nach Infektion mit Leukämie-Viren (S-63) erzeugt, zeigen eine Kreuzreaktion mit den Leukämie-Antigenen aus Rinder- und Menschenserum und Gewebe. Diese Befunde sprechen dafür, dass die Viren-Antigene in den drei Systemen sehr ähnlich sind.

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The Conversion of H^3-Tyrosine to H^3-DOPA in the Adrenal Glands under in vivo Conditions

Recently UDENFRIEND et al.[1-3] were able to demonstrate the formation of DOPA by incubating tissue slices of adrenals and homogenates of adrenal medulla and of brain with radioactive tyrosine. Furthermore they could show the DOPA formation by an in vitro system with purified tyrosine hydroxylase. This paper demonstrates the synthesis of DOPA under in vivo conditions.

Domestic fowl and cats were injected with H^3_2-3,5-L-tyrosine (30,000 mc/mM)[4], 100 mc/kg, intravenously in urethan (1 g/kg) and ether anaesthesis, respectively. The animals were decapitated 20 min after injection. The DOPA decarboxylase was inhibited with 100 mg/kg NSD-1034 (N-methyl-N-(3-hydroxybenzyl)hydrazine dihydrogen phosphate)[5] in some of the animals by i.v. injection 20 min before tyrosine injection. About 1 g of various organs (adrenal glands, brain stem, heart, liver, kidney, spleen, pancreas and blood) were homogenized with 4 ml water, to which was added 1 mg each of carrier tyrosine, DOPA, dopamine, norepinephrine, epinephrine and glutamic acid. The acid soluble fraction was isolated with trichloroacetic acid according to the SCHMIDT-THANNHAUSER method[6]. The isolation of H^3-DOPA was carried out in the following way: After extraction of the trichloroacetic acid with ethyl acetate ester the acid soluble compounds were fractionated by high-voltage paper electrophoresis (buffer: pyridine/glacial acetic acid/water, 4:1:47 v/v, pH 5.1, field-strength 40 V/cm, $t = -8$ °C, 180 min; paper: Schleicher and Schüll 2043 b Mgl., washed). The tyrosine-DOPA-spot was eluated and descending chromatography was carried out in n-butanol/glacial acetic acid/water (4:1:5, organic phase, SO_2-atmosphere; paper: Schleicher and Schüll 2043 b Mgl.). This system separated tyrosine from DOPA. The DOPA-spot was eluated again, and DOPA was characterized by its complex with boric acid in high-voltage paper electrophoresis (buffer: boric acid/sodium hydroxide/water, 155:16:5 g/g/l, pH 8.0, field-strength 80 V/cm, $t = -8$ °C, 60 min; paper: Schleicher and Schüll 2043 b Mgl.). With this procedure it was possible to recover 74.3 ± 3.6% of added H^3-5-L-DOPA. All the solutions were prepared with oxygen-free water. Radioactivity was measured by liquid scintillation counting (Tri-Carb, EX 314)[7,8]. In experiments with cats, specimens of effluent venous blood from the adrenals were taken at different times after H^3-tyrosine injection. This was performed by preparing a sac from v. cava inferior, into which only venous blood from the adrenals emptied. All other veins of this region were ligatured. Details of the methods will be published elsewhere.

The Table shows the content of H^3-DOPA in the acid soluble fraction of adrenals and brain stem of domestic fowl and of cats 20 min after i.v. injection of H^3_2-3,5-L-tyrosine. Radioactive DOPA could be found in the adrenals after inhibition of the DOPA decarboxylase by NSD-1034. In domestic fowl H^3-DOPA represented 3.95% and in cats 5.09% of the total radioactivity. Since H^3_2-3,5-L-tyrosine loses 50% of its tritium label during conversion into H^3-5-L-DOPA, the content of labelled DOPA would be approximately twice as large as in the Table if a tyrosine labelled at a different position were used. Neither in the brain stem nor in any other organ investigated, could radioactive DOPA be detected, not even after inhibition of DOPA decarboxylase. Large amounts of radioactive catecholamines were isolated from adrenals in experiments without NSD-1034. The major part (about 80%) of these catecholamines was H^3-dopamine. The H^3-catecholamine content in adrenals of domestic fowl was significantly higher than in cats. This is explained by the higher percentage of chromaffine tissue in adrenals of fowl[9]. H^3-tyramine was absent from all organs examined (< 0.005 μg/g wet tissue)[10].

The Figure presents the concentrations of H^3-tyrosine and H^3-DOPA (μc/ml) in the venous blood of the adrenals of cat between 1 and 18 min after i.v. injection of radioactive tyrosine. Most of the DOPA formed in the adrenals

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