normotension. In the experimental situation described herewith (DCA hypertension rendered normotensive with CCl_4), we have evidence that the block is at the level of converting enzyme, with the resulting presence of vasoinactive angiotensin I, rather than vasoactive angiotensin II.

Summary. Renal and DCA hypertension was produced in rats, and blood pressure was then reduced to normal levels with CCl_4 injections. The juxtaglomerular (JG) index of these Sprague-Dawley rats was determined. After CCl_4 treatment of the renal hypertensive animals, no significant decline in JG granulation appeared as the blood pressure fell to normal. In the DCA hpertensive and the normotensive rats, CCl_4 induced normotension was accompanied by a large increase in granulation. It is thus apparent that the blood pressure decline induced by CCl_4 is not accompanied by any decrease in renal renin secretion. DCA hypertensive rats rendered normotensive by CCl_4 treatment exhibit a normal blood pressure in combination with a high JG index. This paradoxical situation may be the experimental analogue of the clinical syndrome described by Bartter, and attributed by him to an unknown defect in the renin-angiotensin-aldosterone secreting mechanism. Experiments published elsewhere, suggest that, in our experiments, the defect is caused by an inhibition of the enzyme converting angiotensin I to angiotensin II, so that the normal feed back to the JG cell is prevented.

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Inhibitory Effect of Carcinogenic Aromatic Hydrocarbons on the Splenomegaly of Friend and Rauscher Virus Leukemias.* (32270)

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Inhibition of splenomegaly in Friend and Rauscher virus leukemias is currently being used as a convenient index for testing of antileukemic drugs. To examine the validity of this method, 7,12-Dimethylbenz[a]anthracene (DMBA) and 3-Methylcholanthrene (3-MC), two carcinogenic polycyclic aromatic hydrocarbons (PAH), were selected for use

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in these leukemias. Since 1934, carcinogenic PAH have been known to depress the cells of spleen and bone marrow(1). The inhibition they exert on myelo-, erythro- and lymphopoiesis has been compared to acute radiation damage and the action of nitrogen mustard(2). On the other hand, the result of interaction between chemical carcinogens and oncogenic viruses has usually been interpreted as enhancement of the virus-induced neoplasm by the chemical carcinogen(3).

Materials and methods. A single dose of Friend virus or Rauscher virus in sucrose stabilizer(4) ranging in 10-fold dilutions from 10^{-1} to 10^{-7} for the Friend virus and from 10^{-1} to 10^{-6} for the Rauscher virus was injected intraperitoneally into 2 series of adult female BALB/c mice weighing approximately 25 g. One series was employed for each virus. The dilutions of virus were prepared from 20% spleen homogenates in sucrose stabilizer§. The Friend virus pool used in this study had a titer of $10^{5.3}$ ID_{50}/ml and the Rauscher virus pool of $10^{4.8}$ ID_{50}/ml. For each virus dilution, 3 groups of 5 mice each were treated immediately after inoculation of the virus in the following manner:

Group 1 (130 animals): 100 μg of DMBA or 100 μg of 3-MC, both dissolved in 0.1 ml of corn oil, were injected subcutaneously into the scapular region at weekly intervals.

Group 2 (130 animals): 200 μg of DMBA or 200 μg of 3-MC, both dissolved in 0.05 ml of acetone, were applied at weekly intervals to the depilated skin of the back.

Group 3 (65 animals): These animals received only the virus and were included as controls. Additional controls were treated only with DMBA or 3-MC or only with the sucrose stabilizer.

Each animal was palpated at 2-day intervals looking for enlargement of the spleen. The number of days until the spleen extended beyond the breast line was considered as the latent period of the leukemia. Spleen weight was determined either at time of death of the animal or after 100 days when all experiments were terminated. Spleen, liver, adrenals, and neoplasms of skin and subcutaneous tissues were examined histologically in sections stained with hematoxylin and eosin.

Results. The gross pathological changes were the same in both types of leukemia. However, we did not encounter, in either the treated or untreated groups, ruptured spleens and intraperitoneal hemorrhage as previously described by investigators of Friend virus leukemia(5) and Rauscher virus leukemia (6,7), although in many animals the spleens looked like bags filled with blood. Our observations are in agreement with the recent statement(7) that the histological appearance of spleen and liver is identical in both types of leukemia.

After 100 days, local sarcomas had developed in 49% of the animals which had received DMBA subcutaneously and in 90% of the animals which had received 3-MC subcutaneously. One hundred per cent of the animals painted with DMBA and less than 1 per cent of the animals painted with 3-MC which were alive after 100 days, showed skin changes ranging from small areas of hypertrophy and hyperkeratosis of the epidermis to squamous cell papillomas. Only occasionally were there signs of carcinoma *in situ* or, even rarer, of early invasive squamous cell carcinoma in the animals painted with DMBA. A small number of the animals injected with either DMBA or 3-MC developed epidermal cysts, apparently from pieces of epidermis which were dislodged by the trauma of the injection. A greater number of invasive squamous cell carcinomas developed from these cysts than from the squamous cell papillomas of the skin. The explanation might be a more sustained contact of the carcinogen with the squamous epithelium of the cysts. There were fewer sarcomas and papillomas in the animals which died before the end of the experiment. Leukemia, not the chemically induced neoplasms, was the cause of death in all animals used.

Analysis of variance with a factorial design was performed¶ using spleen weights,

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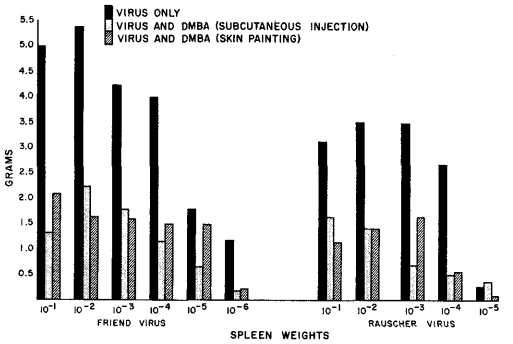

FIG. 1. Effect of DMBA on spleen weight of mice with Friend and Rauscher virus leukemia. (Each bar represents the average for a group of 5 mice. The results for the dilutions 10^{-7} of the Friend virus and 10^{-6} of the Rauscher virus are omitted, since these animals did not develop leukemia.)

rounded to the nearest hundreth gram, of mice treated with DMBA or 3-MC and of control mice, both groups receiving either Friend or Rauscher virus in dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. (Data from mice receiving higher dilutions of virus were excluded from the analysis, since many animals in each group escaped infection). The error mean squares for the spleen weights of all mice infected with Friend and Rauscher virus were 0.92 and 0.67 g respectively. Interaction between chemical treatment and virus dilution was insignificant for either virus. The respective mean squares were used to determine confidence levels for the average spleen weight of individual groups of mice treated with DMBA or 3-MC.

At the 95% confidence level, the average spleen weights of the groups of mice infected with either Friend or Rauscher virus and treated with DMBA were significantly lower in most dilutions than the average spleen weights of infected untreated control mice (Fig. 1) and not significantly different from the average spleen weights of normal control mice of the same age. Similar results were obtained in the groups of mice infected with Friend virus and treated with 3-MC, especially when 3-MC was injected instead of painted (Table I). However, for the combination of Rauscher virus infection and treatment with 3-MC, at the 95% confidence level, the average spleen weights were not significantly different in most dilutions from the infected untreated control mice (Table I) and larger than the average spleen weights of normal control mice of the same age.

Although the average latent periods and survival times in the groups of mice infected with Friend or Rauscher virus and treated with DMBA or 3-MC seemed to be longer and correlated with the spleen weights (Table I), the greater variance of the responses among mice in such small groups made it impossible to show statistically significant differences at the 95% confidence level.

The development of sarcomas and papillomas was unrelated to the results of our experiments. Nevertheless, the almost complete absence of skin changes after painting of 3-MC might be due to poor absorption and also explain the stronger effect on spleen weight, latent period and survival time when this substance was injected subcutaneously. Even so, 3-MC was inferior in its effect to DMBA. Although no difference in gross and histological appearance of the spleen in both types of leukemia could be found, the effect of DMBA and 3-MC was more pronounced in Friend virus leukemia than in Rauscher virus leukemia.

Histological examination of the spleens of the animals eventually dying from the leukemia revealed as cause for the inhibitory effect of DMBA and 3-MC on the development of splenomegaly (a) a lesser degree of hemorrhagic necrosis, and (b) lack of erythroblastosis. Whereas the spleens of infected untreated animals had lost their histological architecture due to hemorrhagic necrosis which expanded the spleen volume by engorgement with blood and almost obscured the proliferation of the leukemic reticulum cells, the spleens of the treated animals were bloodless and composed of the diffusely proliferating reticulum cells. The proliferation of erythroblasts was suppressed in our experiments, but this would explain only a relatively small reduction in spleen size. Erythroblastosis is a characteristic feature of Friend and Rauscher virus leukemias, possibly due to a direct stimulating effect on the spleen(8). This might be abolished by the depressive effect of the chemical carcinogen on the erythroblastosis(1,2).

Discussion. The splenomegaly in Friend

TABLE I. Effect of DMBA and 3-MC on Latent Period and Survival Time, and of 3-MC on Spleen Weight of Mice with Friend and Rauscher Virus Leukemia.

	Latent period in days										Survival time in days									
	Friend virus					Rauscher virus					Friend virus					Rauscher virus				
Dilution of virus	Virus only	DMBA SC	DMBA SkP	3-MC SC	3-MC SkP	Virus only	DMBA SC	DMBA SkP	3-MC SC	3-MC SkP	Virus only	DMBA SC	DMBA SkP	3-MC SC	3-MC SkP	Virus only	DMBA SC	DMBA SkP	3-MC SC	3-MC SkP
10^{-1}	10	14	13	8	10	8	14	53	14	13	40	73	83	46	55	86	59	82	66	47
10^{-2}	10	15	19	16	15	15	33	50	19	20	42	83	71	54	68	47	77	82	61	64
10^{-3}	16	43	60	26	26	30	63	61	33	27	47	77	93	57	77	66	82	82	84	60
10^{-4}	27	65	55	35	33	52	50	98	85	43	52	90	99	54	76	78	84	100	88	100
10^{-5}	50	85	81	99	100	100	94	100	100	100	85	89	94	100	100	100	99	100	100	100
10^{-6}	91	100	92	100	100						91	99	97	100	100					

	Spleen weights (g)					
	Friend virus			Rauscher virus		
Dilution of virus	Virus only	3-MC SC	3-MC SkP	Virus only	3-MC SC	3-MC SkP
10^{-1}	4.99	1.45	3.83	3.17	2.50	2.71
10^{-2}	5.36	1.39	2.52	3.56	2.17	2.66
10^{-3}	4.21	1.76	1.82	3.56	2.34	2.30
10^{-4}	3.96	2.00	2.90	2.76	.46	1.99
10^{-5}	1.77	.26	.15	.26	.18	.19
10^{-6}	1.19	.14	.14			

SC: subcutaneous injection. SkP: skin painting. Each value represents the average for a group of 5 mice. The results for the dilutions 10^{-7} of the Friend virus and 10^{-6} of the Rauscher virus are omitted, since these animals did not develop leukemia.

and Rauscher virus leukemia is a complex phenomenon and consists of at least 3 components. The first of these is a "direct neoplastic effect", namely, the proliferation of the leukemic reticulum cells. The second and third are "indirect neoplastic effects" and consist of (a) hemorrhagic necrosis which plays a great role in the enlargement of the spleen, and (b) proliferation of erythroblasts which contributes only little to the increased spleen size. This distinction is of practical importance, since inhibition of splenomegaly in Friend and Rauscher virus leukemia is used as an index for testing of antileukemic drugs. This by itself is not a reliable index, since it is not possible to separate the "indirect neoplastic effects" from the suppression of the proliferation of the leukemic reticulum cells which the testing is supposed to evaluate. The histological determination of the degree of reticulum cell proliferation in the liver might prove to be a more accurate method, since "indirect neoplastic effects" do not complicate the picture in this organ.

Although the effect of DMBA and 3-MC was pronounced in regard to inhibition of splenomegaly, the apparently prolonged latent periods and survival times also need an explanation. Unless inhibition of splenomegaly *per se* should increase these two in some unknown way, this result of our experiments could only be explained by actual inhibition of the proliferation of the leukemic reticulum cells, *i.e.*, the leukemic process proper.

There is some evidence for a direct inhibitory effect of carcinogenic and non-carcinogenic PAH on established neoplastic cells. Inhibition of growth of the Jensen rat sarcoma(9), of other transplantable rat tumors(10) and of spontaneous mammary carcinomas in mice(11) has been reported. Similar observations were made for the Rous sarcoma in chickens(12), a variety of transplanted tumors in rats and mice(13), and for transplanted hormone-dependent mammary fibroadenomas in rats(14). This experience has justified the occasional use of carcinogenic PAH in human cancer therapy(15). A proposed explanation for these results was that the observed tumor inhibition by the carcinogenic PAH was only part of the general inhibition of somatic growth(16), or that caloric restriction, due to decreased food intake associated with the general toxic effect of these chemicals, might be a factor(17).

The discovery that DMBA induces selective necrosis and hemorrhage of the adrenal cortex of the rat(18) has led in the last few years to a lively discussion of the relationship between adrenal cortical insufficiency and carcinogenesis in general. Special consideration was, therefore, given in our experiments to the histological examination of the adrenals. No changes were observed. Our results in BALB/c mice, together with the observation that of all the carcinogenic PAH only DMBA produces adrenal cortical necrosis and only in the adult rat, but not in mice (Swiss, MA, C57BL, AKR), hamsters or guinea-pigs(19), seems to exclude a general role of the adrenal cortex in this type of carcinogenesis.

Another possible explanation of our results could be the hormone-mimetic effect of carcinogenic PAH. DMBA and 3-MC are both considered as having a progesterone-mimetic effect(20). However, since Estradiol has been shown to inhibit the splenomegaly of Friend virus leukemia(21), one might speculate that a progesterone-mimetic substance would rather enhance it.

So far, there is no evidence that carcinogenic PAH might interfere with the relationship between the leukemogenic virus and its host cell, either by inhibiting the penetration and proliferation of the virus or by decreasing the ability of the reticulum cell to undergo the malignant transformation leading to proliferation.

Summary. 7,12-Dimethylbenz[a]anthracene and 3-Methylcholanthrene applied weekly to BALB/c mice by subcutaneous injection or skin painting inhibited the splenomegaly associated with Friend and Rauscher virus leukemias. The concomitant development of sarcomas and papillomas was unrelated to the result. Since the effect was mainly due to interference with the non-neoplastic components responsible for the splenomegaly, inhibition of splenomegaly in Friend and Rauscher virus leukemias is not considered to be a reliable index for testing of antileukemic drugs. Latent periods and survival times of the treated ani-

mals seemed to be prolonged indicating that a direct antineoplastic effect might play an additional role. The adrenals were not affected by the chemical carcinogens.

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Urinary Protein and Carbohydrate III. Age Differences in Acid Mucopolysaccharides in Human Beings. (32271)

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Urinary acid mucopolysaccharides (AMP) have been of interest to investigators since Brante(1) reported that Hurler's syndrome was an acid mucopolysaccharidosis. At that time, the presence of AMP in urine of normal persons was in doubt. Later, Kerby(2) used Astrup's method(3) to precipitate AMP from normal urine and to determine the normal level of excretion. She also used paper chromatography to prove that chondroitin sulfate was the principal AMP present in normal urine. Heremans *et al*(4), using electrophoretic techniques, demonstrated 3 AMP components in normal urine. Shortly thereafter, both the Dorfman(5) and Meyer(6) groups identified chondroitin sulfate B (CSB) and heparitin sulfate (HS) in the urine in gargoylism (Hurler's syndrome). They(6) also reported that chondroitin sulfate A and C (CSA and CSC) were the principal AMP in the urine of normal children. Recently King (7), using chromatographic techniques, obtained evidence for the presence of HS in normal urine. This finding was confirmed by Di Ferrante(8), Linker(9), Berenson *et al* (10) and Teller(11). The latter authors also believed that in addition to CSA and CSC there were CSB(8,9,10,11) and keratosulfate (KS)(10) present in normal urine. Furthermore, both King(7) and Kelley *et al*(12) revealed the presence of neutral sugar in AMP of urine. The complexity of the composition of urinary AMP and its relation to pathological conditions deserves further investigation.