5. London, I. M., *Bull. N. Y. Acad. Med.*, 1960, v36, 79.

6. Beutler, E., Dern, R. J., Alving, A. S., *J. Lab. Clin. Med.*, 1954, v44, 439.

7. Steinberg, D., Astrow, B. H., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 31.

8. Borun, E. R., Figueroa, W. G., Perry, S. M., *J. Clin. Invest.*, 1957, v36, 676.

9. Levy, L. M., Walter, H., Sass, M. D., *Nature*, 1957, v180, 1211.

10. Sass, M. D., Levy, L. M., Walter, H., *Canadian J. Biochem. and Physiol.*, 1963, v41, 2287.

11. Marks, P. A., Johnson, A. B., Hirschberg, E., *Proc. Nat. Acad. Sci. U.S.*, 1958, v44, 529.

12. Prankerd, T. A. J., *J. Physiol.*, 1958, v143, 325.

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Coombs-positive Hemolytic Anemia and Generalized Amyloidosis in Mice Following Transmission of Subcellular Leukemic Material.* (29478)

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Recently we reported(1) the evidence of a murine leukemogenic virus present in a transplantable line (line 66) of plasma cell leukemia(2). This assumption was based on the transmission of the leukemia with subcellular material, on the finding of characteristic virus-like particles with "tail" with the electron microscope in negatively stained preparations of plasma, and finally on an inhibition of growth of the leukemia following "immunization" with virus extract of leukemic tissue. These findings prompted us to examine several of our other transplantable lines of leukemia for the possible presence of virus particles in negatively stained preparations of plasma. Virus-like particles were found, although in small numbers, in most mice examined, *viz* mice of the following transplantation lines: 3 sublines of line 68(3), namely a plasma cell leukemia without paraprotein in serum, one with gamma paraprotein and a mast cell leukemia, an amyloidosis-inducing reticulosarcomatosis(4), and a leukemia associated with macroglobulinemia(5). Consequently, attempts to transmit these lines of leukemia with subcellular material were carried out in addition to identical experiments using material from line 66 and from the 2 transplantation lines derived from line 66 by subcellular transmission(1). The experimental results using material from these 8 different lines were identical, and they will therefore be described collectively.

Three different *methods* were used for subcellular introduction of leukemic material (Table I). 1. In Exp. 24 0.1-0.2 ml of the supernatant from vigorously centrifuged minced leukemic tissue, spleen or plasma was injected subcutaneously or intraperitoneally. 2. In Exp. 25 0.05-0.1 ml of virus extract of leukemic tissue or plasma, prepared according to the method of Moloney(6) was inoculated along the same routes. In both experiments adult as well as new-born mice were injected. 3. In Exp. 22 attempts were made to transmit the virus through the placenta or with the milk from mice injected with subcellular leukemic material or from mice carrying whole cell transplants of the various above-mentioned leukemias.

The subcellular material was in all cases derived from (DBA/2 × CBA) F_1 mice, in which all our leukemias have developed(7); it was introduced into DBA/2 and (DBA/2 × CBA) F_1 mice; since our leukemias grow only in F_1 mice, those mice of Exp. 22 which were offspring of mice carrying whole cell transplanted leukemias were consequently F_2 mice (Table I). Recently, Balb/C mice were also included in the experiments.

The *results* were unexpected. They were the same irrespective of method of introduction of the leukemic material and of age of the mice at injection. Therefore they will be

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TABLE I.

	DBA/2								(DBA/2 × CBA) F_1							
	Male				Female				Male				Female			
	No. of mice		Survival time (mo) Mean	Min–Max	No. of mice		Survival time (mo) Mean	Min–Max	No. of mice		Survival time (mo) Mean	Min–Max	No. of mice		Survival time (mo) Mean	Min–Max
Experiment 24*																
Dead or killed with amyloidosis	53	95%	9.0	(3–15)	9	24%	12.9	(10–17)	18	46%	10.6	(5–18)	18	38%	15.0	(12–18)
Dead or killed with leukemia	2		10		4			(12–16)								
Dead or killed with ma.C.					3			(12–15)								
Alive with symptoms of disease	1	2%	12		1	3%	12		1	3%	18		5	10%	16.2	(14–18)
Alive, clinically healthy					21		16	(15–18)	20		16.3	(12–18)	25		17	(14–18)
Total No.	56				38				39				48			
Experiment 25*																
Dead or killed with amyloidosis	13	48%	5.5	(5–11)	3	14%	9.6	(7–11)					2		10.5	(10–11)
Dead or killed with leukemia																
Dead or killed with ma.C.																
Alive with symptoms of disease	14	52%	10.2	(10–11)	2	9%	12									
Alive, clinically healthy					17		11	(10–12)	8		12	(11–13)	11		11.7	(11–13)
Total No.	27				22				8				13			
Experiment 22*												$(F_1 \times F_1) = F_2$				
Dead or killed with amyloidosis	7	100%	6.4	(5– 7)					14	50%	14.3	(8–19)				
Dead or killed with leukemia									1		12		1		11	
Dead or killed with ma.C.													3		17	(16–19)
Alive with symptoms of disease																
Alive, clinically healthy					8		12§		13		17.7	(17–19)	25		17.4	(16–19)
Total No.	7†				8†				28‡				29‡			

* Exp 24—Inj of supernatant from leukemic tissue or plasma.
Exp 25—Inj of virus extract from leukemic tissue or plasma.
Exp 22—Untreated offspring from mice inj with (sub)cellular leukemic material (see text).

† Offspring from mice injected with virus extract.
‡ Offspring from mice carrying whole cell transplanted plasma cell leukemias.
§ 4 mice killed 7 mo old (see text); 4 mice are alive.

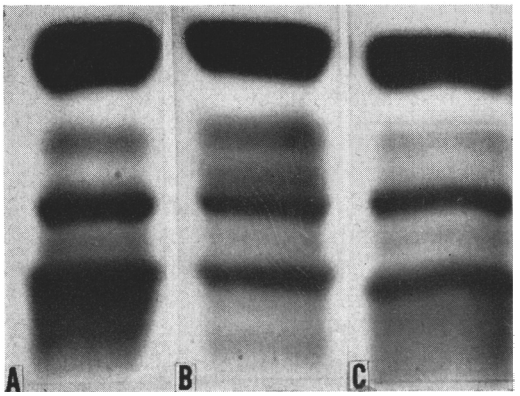

FIG. 1. Electrophoreses on cellulose-acetate strips. a. Serum from Balb/C mouse, injected with virus extract, showing marked hypergammaglobulinaemia. b. Serum from normal (DBA/2 × CBA) F_1 mouse. c. Serum from DBA/2 × CBA) F_1 mouse of Exp. 24 (Table I), demonstrating less marked hypergammaglobulinaemia.

described as a whole. No case of cellular transmission was observed.

The first symptom of the disease in male DBA/2 mice was a slowly developing alopecia on the back, and in male Balb/C mice a roughening of the fur. No such changes were seen in male F_1 mice or in the females of either strain. The next symptom, developing in all male and female mice, was a weight loss, gradually increasing until the final stage, usually 2-4 months later, when the mice were completely deprived of fatty tissue; this was particularly true for DBA/2 males. Simultaneously a hypergammaglobulinemia developed (Fig. 1), shown in immunoelectrophoresis (by Dr. H. Flodgaard, Univ. Inst. of Biochemistry, Copenhagen) to consist of normal gamma globulin with an additional increase of beta-3-1 = complement$_{1\text{-}3}$.

At death an anemia had developed, which became especially severe in DBA/2 males (1.5 mill red cells per cmm were repeatedly observed). This anemia was less marked in DBA/2 females and in F_1 mice of both sexes; in these mice it was furthermore more or less compensated with regard to the number of erythrocytes by an often marked dehydration. Thus, an erythrocyte count of 3.5 mill 18 days before death was found to have risen to 5.6 mill *sub finem*. The anemia was found to be Coombs-positive in all mice tested in the final stage of the disease, irrespective of the strain and sex and the number of erythrocytes. A rabbit anti-normal-mouse-serum antiserum, absorbed carefully with a mixture of red cells from all strains, was used. (The Coombs tests were carried out by Dr. H. Flodgaard and Dr. P. Ebbesen.) Reticulocyte counts often showed normal or slightly increased values; however, in some of the mice with severe or moderate anemia up to 20% reticulocytes were observed. The number of white blood cells was normal or slightly subnormal. The total serum protein level was found increased (up to 7.6 g%) in the dehydrated mice; otherwise it was normal (about 6 g%), also in the mice with severe anemia.

The most characteristic lesion found at death was an often severe generalized amyloidosis. In the spleen (Fig. 2 a) it was seen in all experimental mice with very few exceptions; and in the kidneys (Fig. 2 b) it occurred even as frequently, located interstitially and/or in the glomeruli, the former form often associated with pyelonephritis (papillonephritis) as described in old mice by Dunn(8). It developed diffusely in the liver-parenchyma in some mice reaching extreme degrees (Fig. 2 c), and in the ovaries of all females. It was observed in the intestine, subcutaneous tissue and other organs. The amyloid substance was Congo-red-positive. The thymus and the lymph nodes showed varying degrees of depletion of lymphocytes, and in the latter the medullary cords were often filled with normal, mature plasma cells, more so than were seen in normal mice. Not all lymph nodes showed the same stage of these lesions.

In about 50% of DBA/2 females and in about 75% of F_1 mice of both sexes killed by the disease a characteristic lung lesion was found (Fig. 3), consisting in large cuffs of mature, strongly pyroninophilic plasma cells around the bronchi and vessels in one or two, but not in all lobes. This lesion was associated with varying degrees of bronchitis and pneumonia, which no doubt were secondary to the plasma cell infiltrations. As a consequence of the longer survival time for these groups of mice, *vide infra,* these lesions were found in older mice. Such lesions were

not observed in DBA/2 males.

As is evident from Table I, which includes the total experimental material, 88 or 100% of male DBA/2 mice have succumbed to or are alive with symptoms of the disease (regressions have never been observed), whereas the incidence in 47 F_1 males, in 28 F_2 males and in 158 females does not exceed 50%; however, many mice of these groups are still alive and may develop the disease late in life. The mean and particularly the minimum survival time was much shorter for the male mice than for the females; this was most conspicuous for the DBA/2 males. As indicated in the Table, a total of 8 leukemias was observed in Exp. 24 plus 22, the survival time being 10 to 16 months; it is therefore questionable whether they are induced or spontaneous.

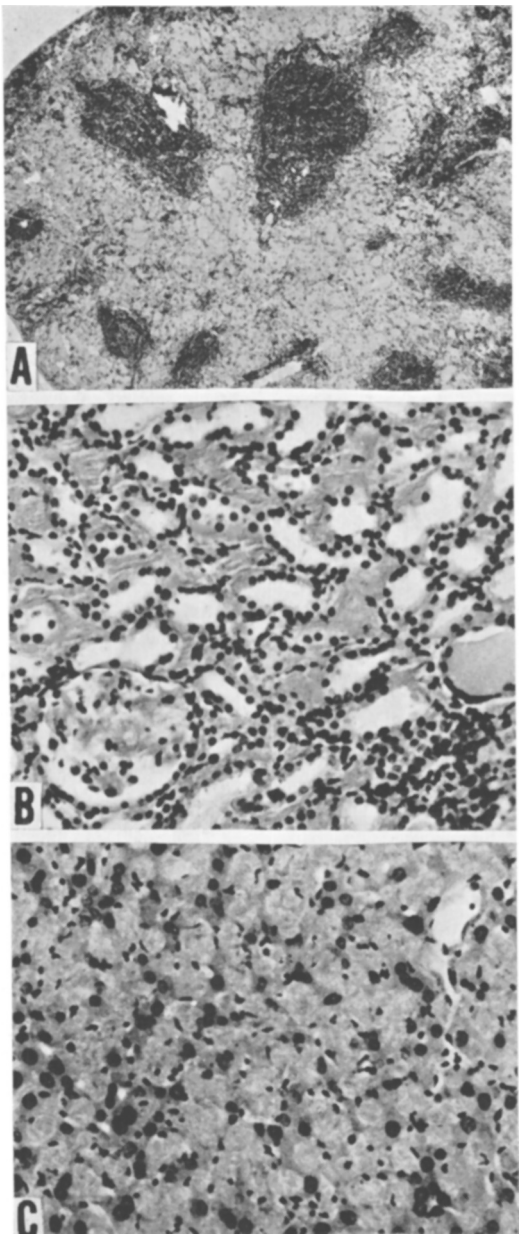

FIG. 2. Amyloid development in a male (DBA/2 × CBA) F_1 mouse of Exp. 24 (Table I). Congo-red staining. a. Spleen with marked amyloid deposits, × 25. b. Kidney with amyloid substance interstitially and in a glomerulus, × 200. c. Liver with marked intercellular amyloid formation, × 200.

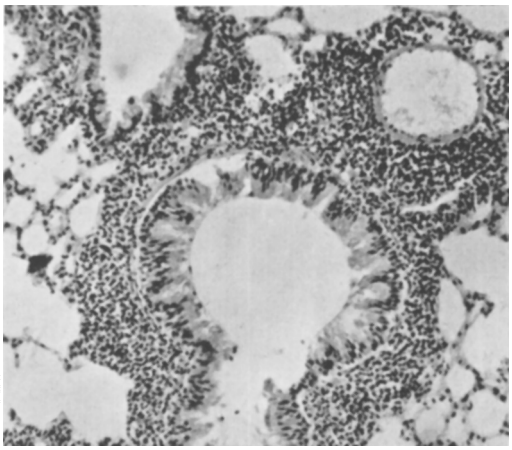

FIG. 3. Lung from female (DBA/2 × CBA) F_1 mouse of Exp. 24 (Table 1) showing cuffs of plasma cells around a bronchus and a vein. Hematoxylin and eosin, × 100.

At present a large number of Balb/C males, but no females show early symptoms of the disease, irrespective of the method of transmission employed; only very few of them have, however, died.

Electron microscopy of negatively stained preparations of plasma from diseased mice (carried out in collaboration with L. Henriksen, Univ. Inst. of Biophysics, Copenhagen) have shown few, but unmistakable virus-like particles with "tail"; this applies also to plasma from 4 female DBA/2 mice of Exp. 22 (Table I), killed when 7 months old in a clinically healthy state and showing no microscopic lesions.

The disease has repeatedly been transmit-

ted to DBA/2, F_1 and Balb/C mice with virus extract or supernatant from spleen and from plasma from the amyloidotic mice as well as from neoplastic tissue from mice with the leukemias and mammary carcinomas developed in Exp. 24 and 22 (Table I). These transmissions have all caused lesions identical to those described here; this applies to incidence and survival time as well as to strain and sex specificity.

Discussion. The etiology and pathogenesis of the disease described above is unknown. The presence of a leukemogenic virus, whether or not it is the causal etiological factor, seems warranted from the development of 2 previously described leukemias in (DBA/2 $\times$ CBA) F_1 mice(1) and of 3 recent leukemias induced in Balb/C mice as the result of subcellular transmission.

A disease in mink of probable viral etiology(9) has been reported to cause hypergammaglobulinemia and kidney lesions somewhat similar to those mentioned here. A virus isolated from a mouse lymphosarcoma(10) has been found to provoke splenic and hepatic amyloidosis and plasma cell infiltrations, particularly in the lungs. Finally, the very late stage of infection with lymphocytic choriomeningitis in mice was found associated with weight loss and amyloid nephritis (11). Thus, various viruses may induce lesions similar to those described here. However, strain specificity was observed with the mink disease only, and sex difference was not described in any of the above mentioned papers.

Based on the now well-established fact that leukemogenic viruses elicit antigenicity of the infected cells and antibody production against these transformed cells the following pathogenesis for the present disease is proposed: an introduced (avirulent?) leukemogenic virus elicits antigenicity of some host cells; this in turn activates immunologically competent cells of the host to respond with antibody production (apparent as hypergammaglobulinemia), possibly with the development of "forbidden clones"(12). Coating of the red blood cells with the produced antibodies may be the cause of the hemolytic anemia. The plasma cell infiltrations in the lungs may represent an expression of the increased demand for antibody production, to some extent substituting the antibody-producing cells of those organs, in which such cells are reduced in numbers concurrently with the deposition of amyloid substance in their sites. The amyloidosis is probably also a consequence of the heavy antibody production analogous to the amyloidosis induced by other types of hyperimmunization(13,14). Thus, *the development of the lesions appears to have been mediated through an auto-immune mechanism created by the antigenic effect of the virus-transformed host cells.*

Summary. Following transmission with cell-free supernatant fluid or with virus extract of leukemic tissue or plasma from various types of murine plasma cell leukemias a disease developed, characterized by hypergammaglobulinemia, weight loss, anemia, marked splenic, renal and hepatic amyloidosis and plasma cell infiltrations in the lungs. The disease was also transmissible through the placenta or with the milk of infected mice. In DBA/2 males the incidence of the disease was much higher (100%), the survival shorter and the anemia much more severe than was the case in DBA/2 females and in (DBA/2 $\times$ CBA) F_1 mice of both sexes. It is proposed that the development of the lesions is mediated through an autoimmune mechanism created by the antigenic effect of the virus-transformed host cells.

ADDENDUM: Dmochowski (Symposium on Current Research in Leukaemia, Cambridge Univ. Press, in press) has reported on the presence of Mycoplasma (PPLO) in the blood of leukaemic mice and in the blood of leukaemic patients, in addition to the presence of characteristic virus particles. We have also recently observed particles, that may be interpreted as Mycoplasma in the blood of leukaemic mice. Some of the lesions described in the present paper may conceivably be caused by Mycoplasma. This may apply particularly to the observed lung lesion and to the Coombs-postive red cell test. The possible presence of Reovirus Type 3 may also be taken into consideration. Studies along these lines are now in progress.

1. Rask-Nielsen, R., *Nature,* 1963, v200, 440.
2. Rask-Nielsen, R., Heremans, J. F., Christensen, H. E., Djurtoft, R., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 632.
3. Rask-Nielsen, R., Christensen, H. E., *J. Nat. Cancer Inst.*, 1963, v30, 743.
4. Rask-Nielsen, R., Christensen, H. E., Clausen, J., *ibid.*, 1960, v25, 315.
5. Clausen, J., Rask-Nielsen, R., Christensen, H. E., Lontie, R., Heremans, J., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 802.
6. Moloney, J. B., *Fed. Proc.*, 1962, v21, 19.
7. Rask-Nielsen, R., *J. Nat. Cancer Inst.*, 1958, v21, 1083.
8. Dunn, Th. B., *ibid.*, 1944, v5, 17; *ibid.*, 1949, v9, 285; *ibid.*, 1954, v15, 573.
9. Leader, R. W., Wagner, B. M., Henson, J. B., Gorham, J. R., *Am. J. Path.*, 1963, v43, 33.
10. Bather, R., Cushing, J., *Acta Unio contra Cancer,* 1963, v19, 411.
11. Hotchin, J., Collins, N., *Int. Congr. Clin. Path. Mexico,* Oct. 1963, p. 125.
12. Burnet, F. M., *Brit. J. Med.*, 1959, v2, 720.
13. Teilum, G., *Am. J. Path.*, 1956, v32, 945.
14. Osserman, E. F., Takatsuki, K., Talal, N., *Seminars in Hematology,* 1964, Peter A. Mischer, Ed.

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Effects of Alpha-methyldihydroxyphenylalanine, Reserpine and Dihydroxyphenylalanine on Pressor Responses to Norepinephrine and Tyramine in Humans.* (29479)

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In recent years the biogenic amines have become a subject of interest in the biological study of mental illness. The clinical relevance of this interest has increased with the availability of substances which affect the synthesis, storage, release, and degradation of these amines. Within this context we have been particularly interested in the question whether psychiatric patients in any of the various diagnostic categories show differential responses to such substances. Preliminary to such work it appeared important to ascertain the effects of several of these substances on selected normal subjects. This paper reports the effects of intravenous tyramine and norepinephrine on arterial systolic blood pressure in 4 subjects pretreated with alpha methyl-dihydroxyphenylalanine (alpha methyl DOPA), reserpine, dihydroxyphenylalanine (DOPA), and under control conditions. A peripheral measure—blood pressure—was chosen as a dependent variable not because it necessarily reflects central events but because it is available for study and is easily objectified.

Alpha methyldopa was of particular interest because of previous observations which showed it to have a paradoxical enhancing effect on tyramine infusion in hypertensive subjects(1). It was also of particular interest to attempt to repeat in humans, in relatively minute doses, the earlier observation of Burn and Rand(2) that the pressor effect of norepinephrine is increased by reserpine pretreatment in animals.

Procedure. Two men and two women between the ages of 19 and 27 were selected for study. All subjects were in good health and were found to be normal on physical and laboratory examination, including a determination of protein bound iodine.

On each of 5 occasions all subjects were hospitalized under controlled conditions on a clinical research ward. On each occasion the blood pressure response of each subject was determined in the following manner. The subject reclined in a comfortable position and a saline infusion was started in the right arm. The intravenous tubing was connected to bottles containing dilute norepinephrine and dilute tyramine which could be administered at will at a desired rate without the subject's knowledge. A blood pressure cuff and stethoscope bell were attached to the patient's left arm. When blood pres-

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