TABLE IV. Non-reversibility of Li^+ Inhibition on Calcium Transport *in vitro* by Na^+.

Group	Solution bathing mucosal surface* 0-30 min	30-90 min	Solution bathing serosal surface* 0-90 min	Ca^{45} S/M ratios†
1	I (Na^+)	—	I (Na^+)	3.8 (3.2 , 4.2 , 5.1 , 2.7)
2	I (Na^+)	I (Na^+)	I (Na^+)	3.2 (3.3 , 3.0 , 3.4 , 3.0)
3	III (Li^+)	—	I (Na^+)	1.7 (1.5 , 1.7 , 1.7 , 1.8)
4	III (Li^+)	I (Na^+)	I (Na^+)	1.0 (.95, 1.22, 1.00, .84)

* *Cf* Table I for composition of incubating solution.
† Serosal/mucosal ratios of Ca^{45} given as the mean with individual values in parentheses.

that sodium ion is not an essential requirement for active calcium transport, and would tend to separate the calcium transport system from several other transport systems.

Note added in proof: After this manuscript was submitted, a report by H. E. Harrison and H. C. Harrison *(Am. J. Physiol.,* 1963, v205, 107) has appeared in which similar conclusions were reached.

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Immunological Studies in Familial β 2-Macroglobulinaemias.* (28709)

M. SELIGMANN, F. DANON AND J. M. FINE (Introduced by B. H. Waksman)
Research Institute for Blood Diseases, Hôpital Saint-Louis, and National Center of Blood Transfusion, Paris.

The recent finding of familial β 2-macroglobulinaemia(1,2) has led us to initiate investigations in relatives of patients with Waldenström macroglobulinaemia (W.M.)(3), multiple myeloma and "paraproteins" of unknown aetiology. Up to now 91 relatives of 30 patients with W.M. have been studied and, in 4 of these families, a familial incidence of β 2-macroglobulinaemia has been found. Certain characteristics of the macroglobulins (M.G.) in these 4 families, with special reference to their immunological structure, are reported here.

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Materials and methods. Sera were sub-

jected to paper, agar and starch gel electrophoresis. Analytical ultracentrifugation was performed in a Spinco E ultracentrifuge at a speed of 59,780 rpm; 2 sera of the same family were studied simultaneously, using a normal cell and a cell with a prismatic window. All sera were studied at 3 different concentrations. Immunoelectrophoretic analysis was performed with different antisera including sera specific for 7 S γ-globulin, β 2 A and β 2 M, in barbital buffer pH 8.2 ionic strength 0.05; sera containing a macroglobulin spontaneously precipitating in agar, were preincubated with cystein chlorhydrate final concentration 0.1 M. Ouchterlony agar diffusion studies were performed in 1% agar in buffered isotonic saline pH 7.2.

The pathological macroglobulins were isolated by zone electrophoresis in Pevikon C-870† (4) and gel filtration on Sephadex G-200. Preparations with a high content of β 2 M from normal pooled human sera and from pooled sera of patients with liver cirrhosis were prepared by similar methods. No attempt was made in these studies to obtain an immunologically pure preparation of β 2 M.

The antigenic types 1 and 2(5) were determined with a potent antiserum to 7 S normal γ-globulin absorbed by a known group 1 or group 2 gamma-type myeloma protein.

Specific antisera were prepared by hyperimmunizing rabbits with approximately 80 mg of the isolated crude macroglobulin (subcutaneous weekly injections in complete Freund adjuvant followed by 3 courses of intravenous injections). These antisera were subjected to 3 types of absorption: *Absorption A:* with an amount of normal human serum and pure 7 S γ-globulin sufficient to remove reactivity with any protein other than β 2 M of normal or pathological sera; *Absorption B:* performed by adding to absorption A an amount of normal and/or liver cirrhosis β 2 M preparation sufficient to abolish the precipitin reaction with β 2 M of normal sera, liver cirrhosis or trypanosomiasis sera and 24 unrelated W.M.; *Absorption C:* performed by adding to absorption A the isolated macroglobulin of another member of the same family until no more detectable reaction occurred.

† Superfosfat Fabriks, Stockholm, Sweden.

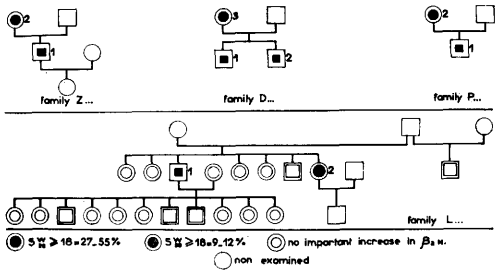

FIG. 1. Familial macroglobulinaemia pedigrees.

Results. Fig. 1 shows the 4 family pedigrees. The patients D1, D2, P1 and L2 had the clinical and haematological features of W.M. The 3 mothers, Z2, D3 and P2 were quite healthy; clinical and haematological examination were negative. Chromosomal studies performed by Dr. J. Lejeune on 3 of the probands showed no abnormality. Some of the siblings of L1 and L2, and some children of L1 had minor modifications of the level of γ-, β 2 A or β 2 M, but none showed an increase in β 2 M greater than twice the normal. No consanguinity was noted in these 4 families.

All 9 pathological sera gave a typical narrow band on paper or agar electrophoresis. None of these M.G. penetrated in starch gel. As noted in Table I, the sedimentation constants of the M.G. in a given family were often but not always similar; the content of serum in γ- and β 2 A-globulins was very variable, even in the asymptomatic mothers with about 10% of heavy constituents.

The antigenic types 1 and 2 appeared to be the same for the macroglobulins in a given family.

Antisera to M.G. D1, D2, P1 and L1 have been studied. After Absorption A (see methods), a spur was formed in Ouchterlony plates, for all these antisera, between the line of the homologous M.G. and the lines given by M.G. of all liver cirrhosis, trypanosomiasis, and unrelated W.M. sera tested. After absorption B, all the antisera still gave a strong line with the homologous M.G.

After absorption A, a spur was formed,

TABLE I

	Age	Total proteins, g %	SIA	β 2 M	β 2 A	γ	Ultracentrifugation °S_{20}^{w}	%
Z 1	55	10.7	++	↗ +++	↘ ++	↘ +	19	26
							29	1
Z 2	85	9.6	—	↗ ++	N	↗ +	?	
P 1	38	9.9	—	↗ +++	N	↗ +	19	43
							28	9
							36	3
P 2	70	8.3	++	↗ ++	↘ ++	↘ ++	19	11
							28	1
D 1	65	13.2	—	↗ +++	↘ +	↗ +	19	33
							28	9
							35	2
D 2	63	12	—	↗ +++	↘ ++	↘ +	19	32
							26	10
							35	4
D 3	85	8.2	—	↗ ++	↗ +	↗ +	19	9
L 1	61	8.2	++	↗ +++	↗ +	↗ +	19	27
							28	4
L 2	54	8.3	++	↗ +++	↘ ++	↘ ++	18	20
							20	10
							28	3

with each of the 3 antisera to P1 M.G., between the homologous M.G. and the mother's M.G. (P2), as well as unrelated M.G. (Fig 2 A). After absorption B, the antisera no longer reacted with P2 (Fig. 2 B). After absorption C with P2, the reaction with P1 remained and the amount of anti-P1 antibodies was roughly the same in B and C absorbed antisera.

Similar results have been obtained with 2 antisera to L1 M.G. After absorption B, they no longer reacted with M.G. L2 and, in addition, no reaction was seen with undiluted sera of all the other relatives. After absorption C with L2, these antisera still reacted with M.G. L1.

The results obtained with antisera to D1 and to D2 are of special interest. After absorption with a great amount of normal M.G. (Absorption B), antisera to D1 showed no evidence of reaction either with 24 unrelated M.G. tested at different concentrations, or with D2 M.G. But they did react with D3 M.G. and the line given by D1 was in continuity with the line given by D3 (Fig. 3). The antisera to D2 M.G., absorbed in a simi-

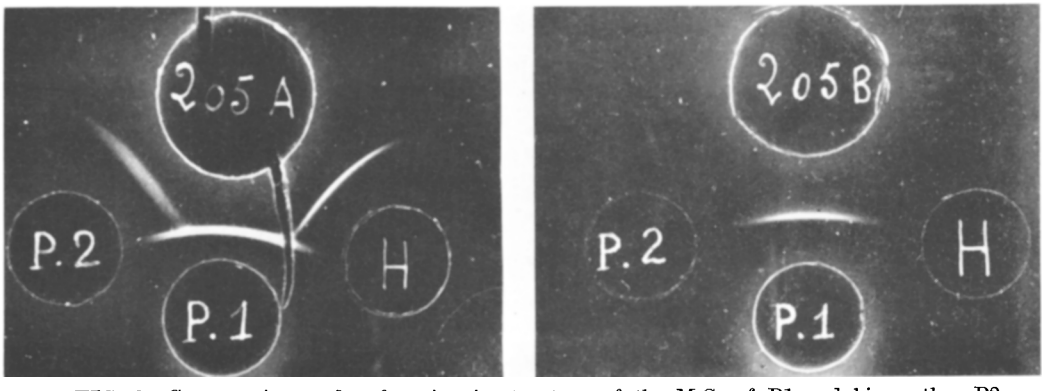

FIG. 2. Comparative study of antigenic structure of the M.G. of P1 and his mother P2 with an antiserum to P1 (No. 205). H = unrelated M.G.

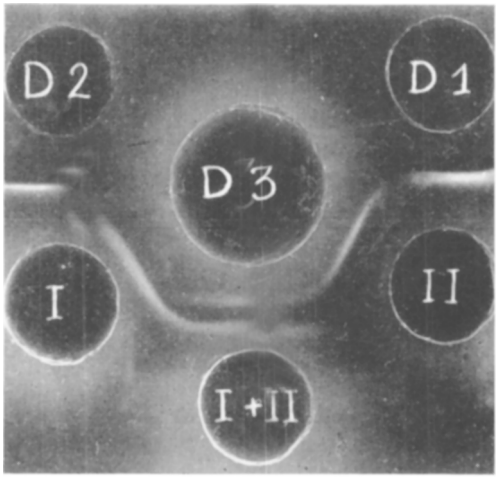

FIG. 3. Comparative study of the antigenic structure of the M.G. of D1, D2, and their mother, D3. D1 and D2 = M.G. fraction 5 mg/ml; D3 = undiluted serum; I = Antiserum to D2 absorbed with normal M.G.; II = Antiserum to D1 absorbed with normal M.G.

lar manner, failed to react with D1 but also reacted with D3 M.G.; no spur was formed between D2 and D3. Fig. 3 demonstrates that the "specific" antibodies to D1 and to D2 react with 2 different kinds of M.G. molecules contained in D3. The same result was obtained with whole D3 serum or the D3 M.G. fraction. When D1 M.G. and D2 M.G. react with a mixture of absorbed anti-D1 and anti-D2, the lines spur through each other. The results of absorptions of C type are the following: anti-D1 absorbed with D2 reacts with D1 and D3 without spur formation; anti-D2 absorbed with D1 reacts with D2 and D3 without spur formation. Anti-D1 absorbed with D3 M.G. no longer reacts with D1. When D3 reacts with anti-D1 absorbed by normal M.G. and with anti-D1 absorbed by D2 M.G., a unique and continuous line is formed.

Antisera to D3 have not been studied.

Discussion. These studies suggest that, at least in some instances of W.M., a genetically inherited disorder is involved. If maternal lymphoid cells are able to cross the placenta (6) the alternative hypothesis of a foeto-maternal chimerism could be discussed, but seems very improbable.

The pattern of inheritance cannot be deduced from the available data. A sex-linked inheritance cannot be ruled out. Whatever the mode of inheritance, the normal or subnormal level of β 2 M in the 11 children (including 8 daughters) of proband L1 suggests that the gene may produce a detectable effect only very late in life.

The experiments with the M.G. of family D clearly demonstrate that the mother's serum contains 2 immunologically distinct kinds of M.G. molecules. The fact that each son possesses one of the mother's M.G. and not the other might suggest a structural gene mutation. But there is a discrepancy between these results and those obtained with families L and P. For example, the "specific" antigenic grouping of P1 is not found in his mother's M.G. This and the low β 2 A-level in some relatives of patients with W.M.(3) rather suggests a control gene abnormality. Therefore these data permit no conclusion as to the nature of the genetic abnormality. This problem is intimately related to that of the truly abnormal nature of W.M. macroglobulins. Further comparative studies on the biochemical structure of these familial M.G. are required.

Summary. A familial occurrence of β 2 macroglobulinaemia has been found in 4 instances. The antigenic structure of the macroglobulins in members of a given family has been investigated with antisera specific to the patients' macroglobulins. In one family, the serum of the asymptomatic mother contained 2 distinct kinds of macroglobulins and each of her 2 sons (affected with Waldenström disease) had one of them and not the other. In 2 other families, the antigenic structure of the macroglobulin was different in each of the 2 relatives.

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Precipitation of Menstruation in Castrated Monkeys with Progesterone in The Presence of Estrogen.* (28710)

FREDERICK L. HISAW, JR.† AND FREDERICK L. HISAW
The Biological Laboratories, Harvard University

Much is known about the physiological conditions under which menstruation occurs in monkeys but its exact cause is yet quite obscure. It has been found that uterine bleeding in a castrated animal usually follows a series of injections of either estrogen or progesterone and that one or more injections of progesterone during an estrogen treatment may be followed by a menstruation. Also, when the 2 hormones are given separately, a minimal dosage is required to prevent "break-through" bleeding during a treatment. These and other considerations have been the subject of a recent review(1).

We have observed on several occasions when a dosage of estrogen was being given which ordinarily was sufficient to prevent "break-through" bleeding, it did not do so when combined concurrently with a small dosage of progesterone. This effect of progesterone on the action of estrogen seemed of sufficient importance to deserve further study.

Methods and results. Two adult monkeys *(Macaca mulatta)* no. 260 and 261, weighing 5354 and 5074 g respectively were castrated and after surgical bleeding and a brief rest period were used in experiments of the following nature. Each animal was given (A) a subcutaneous injection of 10 μg estradiol-17B dissolved in 0.1 cc propylene glycol daily for 15 to 23 days. This was followed immediately by a treatment (B) in which each received 10 μg estradiol-17B and progesterone daily, injected at different sites in 0.1 cc propylene glycol, until bleeding occurred or for a time sufficiently long for it to have happened. Vaginal lavages were taken daily, starting soon after initiation of part "B" of each experiment until its conclusion. After this, the animals were given a rest for a few days to several months before being put on another treatment.

It will be noticed that these animals received 10 μg estradiol daily for the duration of each experiment. It has been found that this dosage of estrogen is sufficient to maintain the endometrium without bleeding for an indefinite period in the great majority of animals. In fact, the 2 monkeys used in these experiments were given 10 μg estradiol daily for 51 days (3-5-60) and uterine bleeding did not occur but did take place on the second day in one and the third day in the other after the injections were discontinued.

The daily dosage of progesterone that will prevent bleeding following discontinuance of 10 μg estradiol daily for about 20 days is approximately 1.0 mg(2). Therefore, the dosages of progesterone used in these experiments were less than this amount, ranging from 0.062 to 0.75 mg. Also, it should be noted that the experiments were distributed over a period of about 4 years, and the animals were used for no other purpose.

Examination of the data shows that bleeding did not occur when 0.75 mg of progesterone was given with 10 μg estradiol, and once in 3 instances when 0.062 mg was given, thus indicating upper and lower limits of dosage required for the reaction. Bleeding with cer-

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† Present address: Dept. of Zoology, Oregon State University, Corvallis.