# Effect of Splenectomy on Cardiovascular Response to Injected Methylcellulose.* (27732)

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Methylcellulose administered intraperitoneally to rats accumulates within elements of the reticulo-endothelial system of the spleen and liver, and is also found within phagocytic cells of the bone marrow, adrenal, ovary, lung and choroid plexus(1). It causes the endothelial and epithelial cells of the kidney to swell and balloon out into foam cells(1,2,6). It also leads to a marked anemia(1) which is prevented by prior splenectomy(4).

Recently we have reported that when it is given either to unilaterally nephrectomized rats subcutaneously or to intact rats intraperitoneally and they are maintained on a high NaCl intake, hypertension, glomerulonephritis, and widespread hypertensive cardiovascular lesions are also produced(5,6). In the intact animals, at least, hypertensive cardiovascular disease is not caused in animals on a low sodium intake(7).

In a more recent experiment we have observed that over the ranges explored methylcellulose caused a splenic enlargement which was almost a linear function of the dose(8). Hence it seemed desirable to determine whether removal of this organ would, perhaps by imposing a greater load upon the renal glomeruli, appreciably affect the response to injected methylcellulose.

*Materials and methods.* Forty-eight young female rats of the Holtzman strain were divided into 4 groups. Groups 1 and 2 were subjected to splenectomy under ether anesthesia and Groups 3 and 4 remained intact. All animals were given a diet of Purina Laboratory Chow and a 1% NaCl drinking solution. On the day after splenectomy, and on days 14, 28, and 30, the animals of Groups 1 and 3 each received 3 ml of a 2% methylcellulose† solution, in 0.9% NaCl intraperitoneally. Animals of Groups 2 and 4 received injections of 0.9% NaCl in the same volume.

Blood pressures were measured periodically by a tail plethysmograph and systolic pressures exceeding 150 mm Hg were regarded as hypertensive.

The animals were killed by ether on the 50th day of the experiment and various organs and tissues were removed and fixed in 10% neutral formalin for subsequent weight and histology. The livers were weighed fresh; the other organs were removed from the fixative, dissected free of extraneous tissue, blotted dry and weighed on an analytical balance. Tissues were stained with Hematoxylin and Phloxin.

*Results.* Blood pressures taken at 9 and 14 days after the first injection of methylcellulose revealed no evidence of hypertension, but at 27 days by which time an additional 3 ml had been given, 2 animals in Group 1 and 5 in Group 3 had pressures above 150 mm Hg. Following administration of 2 additional 3-ml injections on days 28 and 30, a period of 10 days was allowed to elapse before the next pressure was taken. At this time hypertension was noted in over 90% of the animals in Group 1; one animal in Group 2 and in 44% of Group 3. One animal in Group 4 had also become hypertensive. Eight days later the incidence had dropped to 79% of Group 1, and to 21% of Group 3. The incidence in Groups 2 and 4 was found to be 10% and 20% respectively. At the end of experiment there were 11 hypertensive and 3 normotensive animals in Group 1, and 3 hypertensive and 11 normotensive rats in Group 3. One of 10 animals in Group 2 and 2 of 10 animals in Group 4 had also developed hypertension. The one hypertensive in Group 2 displayed a rapidly developing malignant hypertension which, in a period of 3 weeks, had risen from 142 to 216 mm Hg, whereas the 2 in Group 4 had reached 170 and 172 mm Hg respectively. Blood pressure findings are included in Table I.

At autopsy the kidneys and livers of animals which had received methylcellulose were

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† Cellothyl—Chilcott Laboratories.

TABLE I. Effect of Splenectomy on Response to Methylcellulose Injections.

| | | Splenectomized | | Intact | |
|---|---|---|---|---|---|
| | | Methylcellulose | Untreated | Methylcellulose | Untreated |
| Group No. | | 1 | 2 | 3 | 4 |
| No. rats | Initial | 14 | 10 | 14 | 10 |
| | Final | 14 | 10 | 14 | 10 |
| Body wt (g) | Initial | 81 ± 1* | 82 ± 1 | 79 ± 1 | 80 ± 2 |
| | Final | 217 ± 4 | 227 ± 4 | 228 ± 5 | 218 ± 5 |
| Blood pressure (mm Hg) | Day 9 | 124 ± 4 | 120 ± 2 | 124 ± 3 | 119 ± 1 |
| | " 14 | 121 ± 1 | 123 ± 2 | 134 ± 6 | 122 ± 2 |
| | " 27 | 135 ± 3 | 128 ± 2 | 142 ± 5 | 124 ± 1 |
| | " 41 | 166 ± 5 | 136 ± 8 | 151 ± 5 | 132 ± 5 |
| | " 49 | 165 ± 7 | 135 ± 9 | 141 ± 6 | 142 ± 5 |
| Organ wt (g/100 g) | Liver | 7.24± .23 | 4.68± .22 | 6.82± .13 | 4.93± .14 |
| | Kidneys | 1.36± .43 | 1.12± .26 | 1.34± .42 | 1.09± .23 |
| | Spleen | — | — | 1.62± .12 | .28± .07 |
| | Heart | 364 ± 7 | 332 ± 6 | 345 ± 8 | 326 ± 7 |
| | Thymus | 199 ± 7 | 169 ±12 | 177 ± 6 | 168 ± 6 |
| | Adrenals | 37.4 ± 1.18 | 35.4 ± 1.16 | 38.7 ± .71 | 36.8 ± .98 |

* Mean ± S.E. of mean.

visibly enlarged, but were not otherwise distinctively different from those of other groups. The spleens of the intact group given methylcellulose were greatly enlarged. There was, with a single exception, nothing remarkable about any of the other organs examined. The one animal in the group subjected only to splenectomy (Group 2) which had developed a malignant hypertension, however, was noted to have bilateral miliary renal abscesses, kidneys which were double the size of any other animal in the group, and grossly enlarged adrenal glands and heart.

*Organ weights.* Methylcellulose caused a 6-fold enlargement of the spleen. It also caused hepatic enlargement ($P<.001$) which was of slightly greater magnitude among splenectomized than among intact rats, although the difference between the weights on a body weight basis proved not to be statistically significant ($P>.10$). Splenectomy alone had no effect upon liver weight. A statistically significant cardiac hypertrophy was noted among the methylcellulose-treated splenectomized animals as compared with splenectomized controls ($P<.005$). On the other hand there was no such difference between the heart weights of treated and untreated intact animals ($P>.5$). Adrenals and kidneys were also larger in rats treated with methylcellulose although in neither case was this enhanced by splenectomy. The organ weights are given in Table I.

*Histologic findings. Heart.* The heart of one animal in Group 1 had a few foci of inflammatory cells and another from Group 3 had several hyalinized arterioles and a large area of fibrinoid myocardial necrosis. These were from the only 2 treated rats which had attained pressures above 200 mm Hg. The remaining animals showed no conspicuous changes.

*Liver.* Methylcellulose infiltration of the liver was noteworthy. It appeared to be more extensive in the case of splenectomized animals, in agreement with the larger size of these organs and also both foreign-body giant cells and focal accumulations of small lymphocytes were much more common in them.

*Kidneys.* The kidneys of all methylcellulose-treated rats had developed the expected foam cells and, as a consequence, a moderate degree of renal ischemia was evident. There were also interstitial infiltrations of methylcellulose. Hyaline casts were occasionally noted in tubular lumens. There appeared to be no appreciable difference between Groups 1 and 3 as regards the severity of these lesions, and in neither were arterial or arteriolar lesions observed. No lesions could be detected in the kidneys of animals of Group 4 with early salt hypertension. Both kidneys of the one splenectomized animal on salt, which had developed a severe and rapidly develop-

ing hypertension, were found to have multiple abscesses, pyelonephritis and interstitial nephritis.

*Spleen.* The spleen of animals given methylcellulose consisted largely of masses of swollen and confluent reticulo-endothelial cells, multinucleated giant cells and histiocytes, all containing methylcellulose. Lymphoid follicles were compressed, invaded and distorted.

*Discussion.* A previous experiment had shown that whereas a single intraperitoneal injection of 2 ml of 2% methylcellulose failed to cause hypertension or vascular damage, 2 such injections given at a 3-day interval were highly effective. The intermediate dose of 3 ml of a 2% solution used in the present instance failed to produce hypertension within the period in which 4 ml of 2% solution had done so in the previous experiment cited(8). Furthermore given at widely separated intervals in the present experiment, a total of 12 ml of a 2% solution caused in intact animals a lesser incidence and severity of hypertension than had either 4, 6, 8 or 10 ml given over a shorter period. Nonetheless it did produce a greater splenic enlargement. It thus appears that the ability of a given quantity of methylcellulose to cause hypertensive disease is dependent upon the span of time over which it is given while the splenic enlargement is more dependent upon the total dose administered.

Splenectomy appeared to sensitize to the hypertensive effect of methylcellulose, in contrast to the protective effect it is said to exert against methylcellulose-induced anemia(4). Although hypertension appeared to be somewhat later in onset among such animals as compared to intact, a larger percentage of rats were affected in the former group than in the latter. Furthermore, a higher and more sustained elevation of blood pressure was induced in splenectomized rats.

The later onset, however, is puzzling. It may be that removal of the spleen led to hyperactivity of the remaining phagocytic elements of the reticulo-endothelial system. The somewhat greater enlargement of the liver is in accord with this hypothesis, but does not necessarily support it. If such were true the greater sensitivity of splenectomized animals would not become evident until the other defenses were overwhelmed. Although animals with methylcellulosis had larger kidneys than untreated rats, it was also true that hypertensive animals in either splenectomized or intact groups had larger kidneys than normotensive animals of the same group even though they had received the identical total amount of the material. In Group 1 the figures were 1.42 g/100 g body weight as compared to 1.18 g/100 g; and in Group 3 they were 1.53 g/100 g *vs* 1.28 g/100 g. It is difficult to decide whether the kidneys most heavily infiltrated with methylcellulose, and hence most enlarged, were those which set in train a sequence of events leading to renal hypertension, or whether the onset of hypertension, from whatever cause, enhanced kidney enlargement due to methylcellulose. The fact that some animals developed transient hypertension which spontaneously regressed, shows that the disease was labile at first.

Cardiac hypertrophy characterized all hypertensive animals, but only in Group 1 were there enough of these to yield a group average heart weight reflecting significant hypertrophy. The single hypertensive animal in Group 2 and the 2 in Group 4 which became hypertensive also had hypertrophied hearts. It is assumed that the first may be regarded as having developed a renal hypertension, whereas the remaining 2 had begun to develop salt hypertension(9).

*Summary.* Splenectomized animals given intraperitoneal injections of methylcellulose developed a much higher incidence of hypertension than did similarly treated intact rats, although degree of hypertension in both groups was of much the same order of magnitude. The adrenals, liver and kidneys were enlarged to a comparable degree in either group as compared with those of untreated controls. Splenectomized methylcellulose-treated rats, however, developed significant cardiac hypertrophy whereas this was not true of similarly treated intact rats. The results are interpreted as reflecting the more severe damage inflicted by methylcellulose upon animals deprived of one of the organs most concerned with removal of this material from the circulation.

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## Production of L Phase Variants of Bacteria with Cycloserine. * (27733)

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Available evidence indicates that cycloserine acts by interfering with cell wall biosynthesis. Biochemical studies have shown that this antibiotic causes accumulation of N-acetylaminosugar(1), inhibits incorporation of lysine into cell wall fractions(2), and inhibits alanine racemase and alanyl-alanine synthetase in *Staphylococcus aureus*(3,4,5). Nonetheless, cycloserine may affect mammalian cells as demonstrated by its clinical toxicity(6) in contrast to penicillin which clearly acts by specific inhibition of cell wall mucopeptide formation in growing bacteria. Michel and Hijmans were able to obtain L growth from a single strain of streptococcus by exposing the bacterium to cycloserine but stated that the L form was inhibited by low concentrations of this antibiotic(7). Since L forms of bacteria represent the organisms which are capable of growth and reproduction yet lack a rigid cell wall(8,9,10), the L forms should be resistant to cycloserine action if indeed this antibiotic exerts a primary and specific action on cell wall formation. Therefore, we have studied the ability of cycloserine to induce L phase growth of bacteria. The cycloserine susceptibilities of L phase variants derived by exposing bacteria to penicillin have been compared with L phase variants obtained with cycloserine. Also the penicillin sensitivities of L forms derived with cycloserine were compared with those obtained with penicillin. The susceptibilities of the bacteria from which the L forms were isolated were determined and compared with the sensitivities of the L forms. The sensitivities of selected strains of *Mycoplasma* to penicillin and to cycloserine also have been compared.

*Materials and methods.* Ninety-six bacterial strains isolated locally were studied (Table I). They were identified by the usual cultural and serologic technics. Nine strains of *Mycoplasma* were examined.† The L phase variants were isolated on agar plates (Oxoid sensitivity test medium)‡ containing 3% NaCl and 20% heated human ascitic fluid. Penicillin (1,000 U/ml) or cycloserine (1,000 μg/ml) was placed in wells on plates streaked with the test organism. The plates were incubated at 37°C semianaerobically by the Fortner method(11). L phase growth was isolated from the zone of inhibited bacterial growth after 4-7 days incubation and was maintained by serial agar block transfer on agar plates containing penicillin (500 U/ml) or cycloserine (500 μg/ml). The *Mycoplasma* were maintained by serial transfer on PPLO agar (Difco) containing 20% heated human ascitic fluid.

Antibiotic susceptibilities were determined

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† The Preston, 4430, Washington, "L" and C 15 strains were obtained from Dr. L. Dienes, Boston, Mass. Goat and bovine strains were obtained from Dr. H. R. Adler, Davis, Calif., and the L4 strain from Dr. Kuzell, San Francisco, Calif. The "Cont." strain was isolated from rats and showed cultural and virulence differences from the L 4 and Preston strains.

‡ Obtained from Consolidated Laboratories, Inc., Chicago Heights, Ill.