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SOME ASPECTS OF METABOLIC DISEASES IN MAN AND ANIMALS

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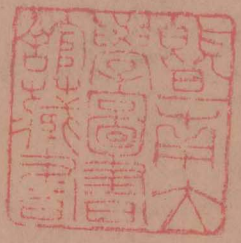

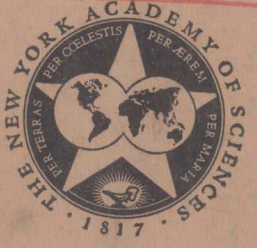

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SOME ASPECTS OF METABOLIC DISEASES IN MAN AND ANIMALS*

CONTENTS

* This series of papers is the result of a conference entitled *Some Aspects of Metabolic Diseases in Man and Animals* held by The New York Academy of Sciences on May 14 and 15, 1962.

Part III: The Porphyrias

Part IV: Ketosis

INTRODUCTION: THERE IS BUT ONE MEDICINE

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Interest in comparative medicine has been growing steadily for a number of years. Theobald Smith, a physician, made a clear statement on the significance of the concept of comparative medicine in a lecture before the Pathological Society in Philadelphia at the turn of the century.

> Man frequently attempts the conquest of problems from the least accessible quarter. This is true of that state of development of medical science when the human species was the chief object of study. Within the past two or three decades medicine has been gradually and almost unconsciously drifting toward animal pathology as the chief, if not the sole, means of clearing up the greater doubts which a broader science inevitably brings with it. We have today definitely reached this second stage, the study of the more accessible, more varied diseases of higher animals. The preparations for this step date well back, and the most conspicuous investigators in human pathology have always had a yearning toward the rich field of animal pathology.

This statement is as true now as it was then. At the time, it may have sounded revolutionary, but today this idea has gained fairly complete recognition, particularly among research workers.

Bacteriology dates from the time (1876) when Robert Koch, a physician, proved that anthrax in cattle was caused by a microorganism. It was discovered only later that this same organism was responsible for the disease in humans, and that—in fact—humans could get the infection from animals. Pasteur, a chemist, found for the first time that bacteria could be attenuated to the point where they would induce protection in animals and man against the same organism, without causing overt disease. "Killed" vaccines were shown in 1886 to induce immunity as the result of cooperative efforts of Salmon, a veterinarian, and Smith, a physician. Another "first" was the discovery around 1888 of a protozoan parasite as the causative agent of Texas Fever in cattle by a team of veterinarians and a physician (Salmon, Kilborne, Cooper, Curtice, and Smith). Moreover, they demonstrated that the disease could be transmitted by an arthropod vector. These findings stimulated Ross in India to achieve the first great break-through in malaria: the discovery of the causative organism and the anopheline transmission of the disease.

Early in this century the fruitful cooperation of these branches of science in elucidating the nature and properties of the viruses of foot-and-mouth disease, fowl sarcoma, and various other diseases initiated the science of virology—another infant that has grown into a giant.

Recently, with an increasing interest in the pathogenesis of organic and other diseases and in the alterations in metabolic processes during the course of these diseases, cooperation between the various disciplines (medicine, veterinary science, and biochemistry) has yielded immense dividends. Results obtained in investigating problems in one species have often opened up new fields of interest in other species and shed new light on *their* disease processes.

Although articles appear in a miscellany of journals all over the world, it was believed that a few selected subjects in the area of metabolic disease could be discussed profitably on a comparative basis in the form of a semi-international symposium at The New York Academy of Sciences. It was hoped that the publication between the covers of a single monograph of the results of some new thinking in the field of metabolic malfunctions would contribute materially to the better understanding of the mechanisms involved and to the re-evaluation of perhaps fondly held ideas on these processes.

KINETIC BASIS OF LIFE PROCESSES: PATHWAYS AND MECHANISM OF HEPATIC PROTEIN SYNTHESIS*

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In the opening paper of a conference devoted to metabolic diseases it may appear startling to state: "Disequilibrium is a condition of life." In several of the problems which are to be presented, pathology is but an extension of physiology.

From the viewpoint of the biochemist and biologist, metabolic processes and metabolities are expressions of a living system. Adherence to a commonly accepted or usual pattern of metabolic performance has been regarded as "normal"; departure or dislocation from this loose base of reference has been viewed as "abnormal." Actually, little separates these alternatives, since each remains an expression of life. Health and disease are partners, or at the least close relatives.

Living processes have altered but little from their primordial characteristics, but thought about them, especially more recently, has been revolutionized. The living body, with its constituent organs and their cellular components, in the present perspective is an *open* or *flow* system. I have discussed the characteristics and metabolic consequences of life as an open system elsewhere.[1] Some qualifying corrolaries are:

1. Such systems are not characterized by classical thermodynamic equilibria. Indeed, they are activated or triggered by departure from equilibrium.

2. *Change* is a prime requirement. "The open system exhibits only the *constancy of constant change. When change ceases, life ceases.*"[1]

3. In flow systems multiple steady state levels of metabolites (as in the blood plasma) are possible, and are an expression of the relative kinetic rates of inflow and outflow.

4. Such systems are characteristically dependent, often crucially, on their external environment. This has necessitated a readjustment of perspective with regard to a number of ancillary concepts associated with classical homeostatic theory.[1]

5. The environmental dependence of the open system is a consequence

* Much of the work reported in this paper was supported by research grant A-831, National Institutes of Health, United States Public Health Service.

of genetic conditioning of both its anatomical and chemical architecture. The body must adjust to life within the confines of its genetic prison.

6. The lack of freedom from the external environment is colinked with inherited *imperfection* of structure or mechanism. Striking examples include such phenomena as the "normal" body's relative disability for the excretion of uric acid[1] and iron[2] and such fundamental considerations as the relative incapacity for the storage of carbohydrate, leading to a crucial dependence for an external source of this commodity. Reorientation, guided by this perspective, indeed counsels the "*need to spare carbohydrate.*"[1] Carbohydrate can be spared by the use of fat—a dominating pattern in metabolic channeling as the normal awake from their normal slumber each morning. Hence, in man—cows are useful but unreasonable creatures—ketosis, an obligatory concomitant of fat utilization, is a normal way of life.[1] Ketonemia of different degrees is merely a reflection of numerous stationary levels possible in a flow system governed by the relative rates of hepatic production and peripheral utilization and urinary spillage. This statement of viewpoint may be provocative of debate.

7. Survival, in the face of imperfection, depends upon the channeling of metabolism along alternative pathways. The availability of such alternatives is itself genetically conditioned. In several so-called abnormal situations the alternative route is not an optimal solution. Its choice has the character of compulsiveness and need not be "rational." However, it does avoid immediate catastrophe. On this basis I have proposed a new concept of the development of metabolic disease states in which the body behaves as though it chooses the alternative of a tolerable disease (permitting survival, albeit at a low level) to a potentially lethal one (FIGURE 1).[1]

8. In a broad and metabolic sense cellular life-span is closely correlatable with the life-span of certain cellular components or metabolites. Thus, the $\tau\frac{1}{2}$ of cytochrome *c* in liver may be as representative of the turnover time of the liver cell, as is the $\tau\frac{1}{2}$ of hemoglobin of that of the red blood cell.[3] On the other hand, the multiple metabolic reactions which occur side by side in cells require "turning on" and "shutting off." The regulation or control involved in these intimate cellular processes is at present a very fundamental problem. Frequently in such reactions a common metabolite may be directed along two or more genetically available alternative pathways, all desirable. The rate of movement and the degree of participation of a common metabolite along one or other route become important parameters in a kinetic approach to understanding. The kinetic rate factor may itself be under genetic control.

9. *Body size-conditioned parameters: metabolic rate factor.*[1] Our studies are on the rat, but we are convinced that the findings are closely applicable

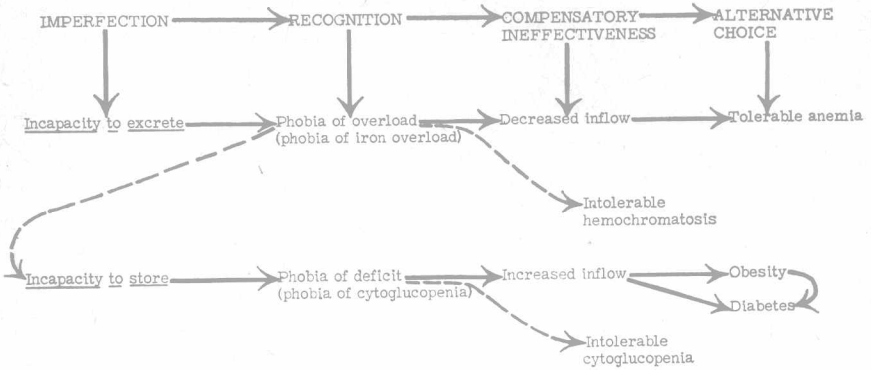

FIGURE 1. Scheme of a sequence in the development of metabolic diseases, which represents the establishment of new (and not necessarily favorable) stationary states. The body behaves as though it chooses the alternative of a tolerable disease to a potentially lethal one, favored by the inherent imperfection of its structure and mechanisms.[1]

to man, about 350 times larger in body size. However, at the outset it is desirable to have a clear comprehension of species (or body-size) conditioned parameters. Dynamically, metabolic processes in the rat go at rates some 4.6 times faster than those in man.[1] The *metabolic rate factor*, 4.6, reflects the degree of difference between the adult rat and adult man in rate per unit time and per unit mass of such measurable quantities as the basal metabolic rate (oxygen consumption or caloric exchange), the outflow of nitrogen in the urine, or of protein production.[1] To an extent the inverse relationship applies to the life-span of the two species—an expression of the philosophical idea that the size of bodies is conditioned by the quickness of life, and that fast life and small size go together, or that enlargement in mass and increase in life-span go hand in hand with a reduction in the metabolic rate factor.[1] On a broad interspecies scale, this, too, represents a choice of alternatives.

In both rat and man the total body protein is about 18 per cent of the body mass, which corresponds with 0.18 gm. protein per gm. mass or 0.029 gm. protein N per gm. mass. In the adult of each species the liver forms the same percentage of the body mass (rat, 2.6 per cent; man, 2.3 per cent). In each the concentration of tissue proteins is the same and the concentrations or stationary state levels under "normal" conditions of the proteins of the plasma and of the red cells are the same. These superficial similarities have been misdirecting, since in a proper orientation it must be stressed that the dynamic maintenance of the same steady state levels, as in the case of the plasma proteins, requires in the *open* biological system rates of inflow and outflow 4.6 times faster in the rat than in man.

10. *Functional potentials and metabolic capacity.*[1, 4] Provision for an adaptive adjustment of concentration levels of certain metabolically active substances to usual functional needs of tissues and bodies is a concomitant of the dynamic stabilization inherent in open systems. Thus, as an example, the normal concentration levels of cytochrome *c* appear to be correlatable not with an ill-defined metabolic "minimum," but rather with *metabolic capacity.*[1, 4] On the other hand, maximal states can be tolerated for brief periods only,[1] and in open or flow systems the regulation of kinetic rates of metabolic processes, their adjustment to usual functional needs which may be appreciably below their maximal potentials, must be of paramount importance. It would be an extravagance of mechanism to maintain the system, except in emergency, at utmost capacity. Analogously with electronic power amplifiers, stabilized through inverse feedback, regulation may break down and aimless cyclicity and oscillation may be invited.

As an extension of concept, suggested by these introductory statements, evidence will be reviewed on certain features in metabolic channeling processes involved in the construction of proteins by the liver. Is it presumptuous to state that knowledge of proteins may be pertinent or basic in many of the considerations within this monograph? Emerging viewpoint in our work has been guided by findings secured by means of two experimental probes, the use of which in metabolic studies was pioneered in our laboratory—the body's repair of a tissue deficit following partial hepatectomy[5-8] and its response to protein deficit emergency in an immunochemically induced nephrosis.[9-12] More recently, as an outgrowth of our earlier work,[13-15] attention has been directed to the study of the regulatory mechanisms in protein biosynthesis in cell-free (microsomal-supernatant) systems, which will be discussed in the following.[16] Important contributions in our joint effort have been made by my colleagues Julian B. Marsh and George A. Braun.

Experimental Methods and Orientation

Partial hepatectomy.[1, 4, 5] The rat has a multilobed liver, and closely reproducible relationships exist between total liver mass and body weight in rats of the same size or age, as well as in the mass of each of the three major lobes and total liver mass. The surgical removal of significantly large portions of the liver is quite easily and quickly performed, and may be described as liver lobectomy. Usually two thirds of the liver mass are excised (FIGURE 2). On a high (31 per cent) protein diet complete restoration of the original liver size, by means of cellular proliferation or regeneration, takes place within three weeks. During the regenerative

period, the amount of new tissue restored or the change in any tissue constituent or metabolite can be determined quantitatively. This was the basis for the introduction of partial hepatectomy as a metabolic technique.[5-8] Early after liver lobectomy, before significant tissue restoration occurs, there is an increase in cytochrome *c*, RNA, and DNA.[6] Indeed, this was the first demonstration in mammalian tissue of a probable relationship between RNA and protein construction. It was postulated that cytochrome *c* and RNA "triggered" protein biosynthesis.[6] In more sophisticated terms, there is a need for energy, as well as for the genetic information and mechanism of protein fabrication, involving several distinctive RNA's—transfer, template, and possibly messenger RNA. Hence, partial hepatectomy has potentialities not only for the study of metabolic reactions, but also for the elucidation of mechanism itself basic to the "how?" of cellular proliferation.

Other findings in our partially hepatectomized rat, which may have

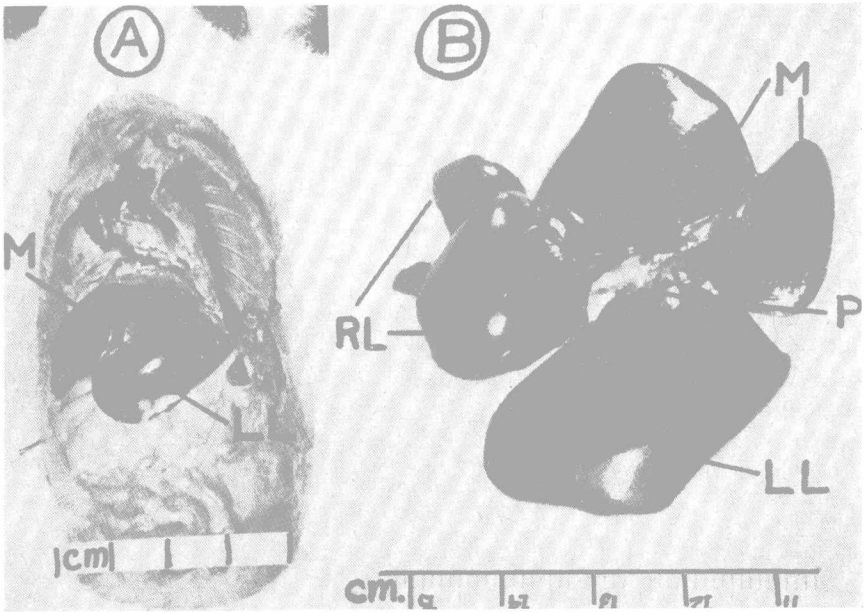

FIGURE 2. Structural relationships of rat liver. *A*, liver *in situ*. *B*, whole liver excised with notched median lobe (*M*) turned back. In the usual partial hepatectomy procedure, ligatures are applied to the pedicles (*P*) of the left lateral (*LL*) and median lobes, which are removed, leaving the associated right lateral and caudal lobes (*RL*). The linear dimensions, applicable to a rat of 250 gm. weight are indicated by the cm. scales.[4] In fasted rats, lobes *LL*, *M*, and *RL* are 36.2, 32.2 and 31.6 per cent respectively of the total liver mass. A standard deviation of ±0.2 applies to each of these mean values and reflects the high degree of reproducibility.[1] Thus, with the excision of lobes *LL* and *M*, the amount of tissue retained is deduced reliably.

relevance to the problem of protein synthesis in the animal body, are the following: (1) Liver regeneration was markedly inhibited on a diet restricted to 5 per cent of protein. Nevertheless, under these conditions, the early post-operative increase in cytochrome *c* and RNA still occurred.[6] Moreover, and rather remarkably, when such rats were switched to high protein the restorative process went into full swing with vengeance, resulting in regenerative overshoot. This phenomenon of regeneration to appreciably above the expected normal liver size was observed only in this group of experiments. Presumably, this is a case of a prepared mechanism waiting to assert itself. The mechanism requires the necessary substrate, exogenous amino acids or protein, to exhibit its potentialities. Or, is it far less simple than this? More recently,[14] we have found that ill-nourished rats, placed on a high protein conditioning diet for some days prior to partial hepatectomy, exhibit more rapid regeneration than that observed consistently in our earlier work. (2) The poorest liver regeneration was seen in rats partially hepatectomized while in a thyrotoxic state induced by administered thyroxine.[7] In such animals the cytochrome *c* and RNA in liver did not increase, and, intriguingly, there was no enlargement of the cytochrome *c* content in other body tissues, with the exception of the kidneys. This was in contrast with the finding of increased amounts of cytochrome *c* in all tissues examined in intact rats with induced hyperthyroidism.[7] Interestingly enough, bilateral adrenalectomy favored the regenerative process in partially hepatectomized rats on a high protein diet.[8] In the days of innocence the pronounced impairment of liver regeneration in hyperthyroidism was ascribed to the heightened metabolic demand of all the tissues, thereby interfering with the diversion of materials necessary for hepatic proliferation.[2, 7] The innocent are doubtless naive, but they may be right. In this day of preoccupation with mechanism at its most fundamental level, it is well to remember that humoral factors still remain involved in the regulation of metabolic processes.

Some comprehension, though perhaps inadequate, of the job to be done in restoration after partial hepatectomy may be gained from the following: a 200-gm. rat in the nonfasted state has 6.25 gm. of liver tissue. 4.3 gm. of liver are removed by the excision of the left lateral and median lobes (FIGURE 2). In terms of protein, a tissue protein deficit of 770 mg. is thereby created. Under conditions of rapid liver regrowth 3.9 gm. of liver tissue may be restored in 6 days. This is at a rate of 0.65 gm. of liver per day or 117 mg. of liver tissue protein per day. Actually, as will be seen later, the daily replacement rate of the plasma proteins (produced in the liver) is 670 mg. per day or nearly 6-fold greater than the amount of protein built into new liver. Such an adult rat consumes 2.1 gm. of

protein daily on a 25 per cent casein diet, and the protein deposited in the regenerating liver per day represents only 5.6 per cent of the total protein intake. These evaluations are consonant with the presumption that partial hepatectomy, under conditions of adequate dietary protein inflow, is a well-tolerated situation quite within the rapid restoration capabilities of the organism. However, function is not maintained at pre-operative levels during liver regeneration, as reflected by a moderate hypoalbuminemia of the order of 18 per cent.[14]

Experimental lipid nephrosis.[9-12] Nephrosis, produced in the rat immunochemically by means of antikidney sera, perhaps represents the closest mimicry of any experimental disease state to a clinical disease entity. The cardinal signs of pronounced proteinuria, severe hypoproteinemia (hypoalbuminemia), hyperlipemia, and edema are all evoked. Pathologically, the induced nephrosis is remarkably similar to the "pure" form of human lipid nephrosis, which is but rarely seen in man, since secondary complications and further renal involvement are almost invariably present in deteriorated human subjects usually studied.[12, 15] This is an advantage of the experimental approach. In both the experimental and "pure" form of the human disease the lesion is confined to the *filtering membrane* of the renal glomeruli (FIGURE 3).[17-21]

In our laboratory we have succeeded in preparing highly potent antirat kidney sera, using not only the rabbit (historically employed for this purpose), but also sheep and guinea pig. From the latter species high potency antisera can be conveniently obtained after relatively short periods of immunization, aided by the Freund and McDermott adjuvant method.[22] The antisera from rabbit, sheep, and guinea pig produce the same experimental disease. However, curious strain specificities have been uncovered. Thus, antiserum produced in immune response to antigen from the kidneys of the Long-Evans strain of rats produced typical nephrosis in the Long-Evans rats, but a rapidly lethal (within 24 hours) hemorrhagic glomerulonephritis in Wistar strain rats. On the other hand, antiserum to the antigen from the kidneys of Wistar rats induced typical nephrosis in animals from both the Wistar and Long-Evans strain (TABLE 1). (It is desirable and of interest to point out that the responsible antigen is not confined to a constituent of the glomerular filtering membrane. Rats, given antisera prepared by immunization against kidney antigen, pass through a very transitory pulmonary reaction. This led us to prepare an antiserum against *rat lung antigen*, using the guinea pig. Remarkably, this antiserum induced typical experimental nephrosis.[24] This observation may have some bearing on etiological factors involved in some cases of the human disease.)

With effective antisera, depending on the amount administered, two

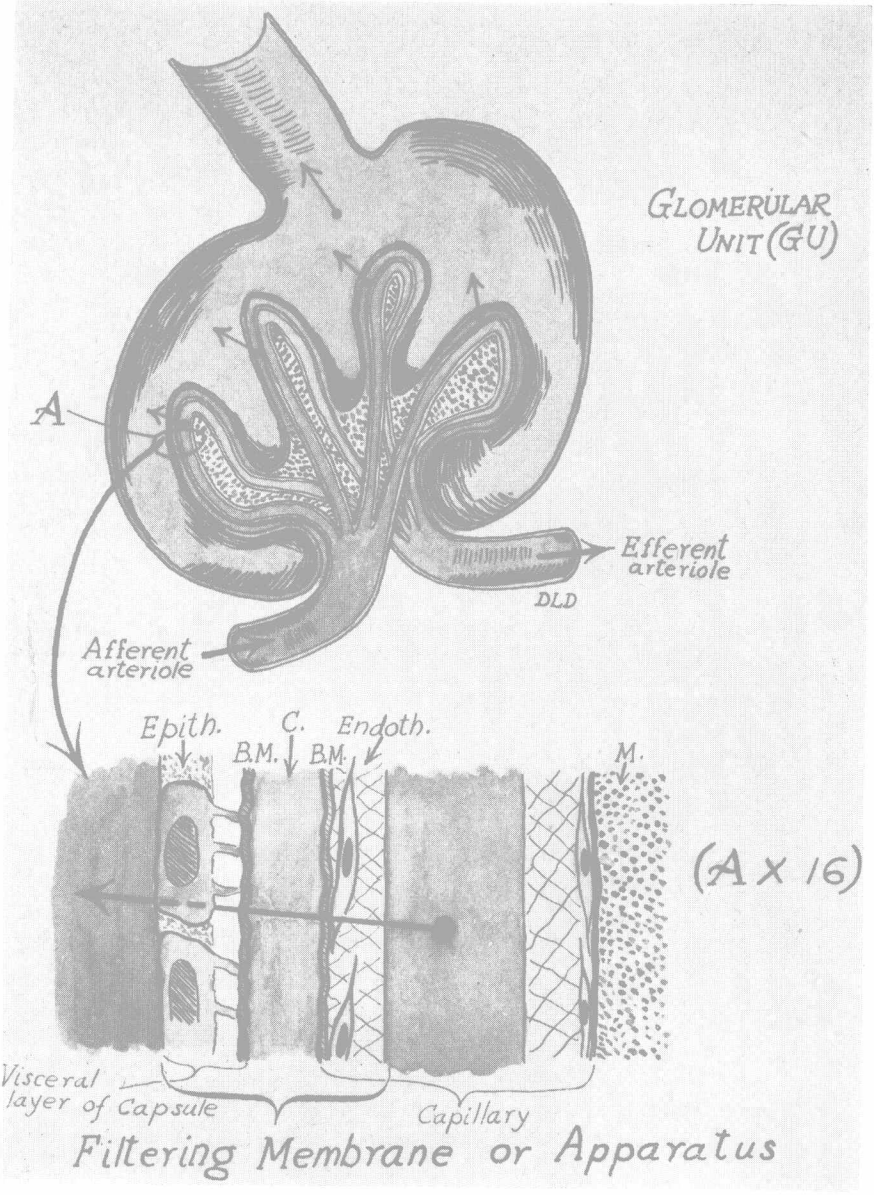

FIGURE 3. Portrayal, based on best recent information,[12] of the architecture of the renal glomerular unit (upper), with an enlarged projection, showing major identifiable structural components of its complex *filtering membrane* or *apparatus* (lower). The glomerulus consists of a capsule (Bowman's), which is an expanded blind end of a proximal tubule, and an invaginated tuft of arteriolar capillaries, whose total length may be some 600 times greater than the diameter of the tuft, thereby providing

TABLE 1
STRAIN SPECIFICITY IN PRODUCTION OF EXPERIMENTAL NEPHROSIS IN THE RAT[23]

Source of antigen	Nephrosis in rat strain	Hemorrhagic glomerulonephritis in rat strain
L-E	L-E	
L-E		W
W	W	
W	L-E	
S-D	S-D	
S-D		W

L-E, Long-Evans strain; W, Wistar strain; S-D, Sprague-Dawley strain.

grades of nephrosis may be produced at will. One is a mild form of the disease, compatible with relatively long survival. In this type symptom-free recovery for moderately long periods, though not necessarily "cure," may take place. The other is a most severe, fulminant form of nephrosis in which a proteinuria of prodigious magnitude may be evident within 24 hours and the signs of protein-deficit emergency, edema and hyperlipemia, as early as 3 days after antiserum administration. In this type of the experimental disease survival is rarely longer than 9 days. Terminally, such animals are depleted to the point of exhaustion. Protein metabolism has been so channeled as presumably to disfavor gluconeogenesis, reflected in potential or frank hypoglycemia.[9] In essence we are dealing with a *protein diabetes*.[9, 12] It is my impression that in contrast with the fatal form of the human disease, death is not uremic, owing to extension of renal pathology to the tubules, with resultant nitrogen retention.

The damaged glomerular-filtering membrane permits losses from the

a relatively enormous filtration surface per functional unit. The filtering membrane is the renal portal of exit from the body, and is thus a crucial point of contact between the body and the environment. Its complexity of structure derives from its two-fold embryological origin. Its outer wall or *environmental* side (a reticulum of specialized epithelial cells, *Epith.*, and their basement membrane, *B.M.*) is the visceral layer of the capsule, whereas its inner wall or *body* side is contributed by the capillaries (endothelial reticulum and cells, *Endoth.*, and their basement membrane, *B.M.*). The two basement membranes are separated by a cementlike substance, *C*, probably of glycoproteins. The capillary tufts are imbedded in a type of connective tissue, the mesangium, *M*.

On the basis of glomerular counts, it is of interest that the number of glomeruli per gm. kidney are 31,000 in rat and 7,000 in man. The ratio of glomeruli per gm. kidney in the two species = 4.4, a value strikingly similar to the metabolic rate factor of 4.6 (see text).

body of extraordinary amounts of the plasma proteins. An appreciation of the magnitude of daily loss of protein may be gained from the fact that a severely nephrotic rat of 75 gm. spills 330 mg. and one of 200 gm. spills 870 mg. of protein per day in the urine.[9] 330 mg. (for the 75 gm. rat) represents 18 per cent of the 1.8 gm. of dietary protein intake. 870 mg. (for the adult rat of 200 gm.) is nearly 50 per cent of the dietary protein, since consumption in the adult is not materially different from that in a young, growing animal. This loss of protein may be contrasted with the appreciably smaller demand upon protein for liver tissue regeneration after partial hepatectomy, but, as will be evident, does not adequately evaluate the metabolic dislocation in the severely nephrotic rat.

Several aspects of the urinary protein loss may afford better insight.

1. As the electrophoretic diagrams (FIGURE 4) of normal rat plasma and of nephrotic rat urine reflect, plasma albumin is the major, but not the only, protein spilled. In the first 24 to 48 hours of the experimental disease, 90 per cent of the excreted protein is plasma albumin (*i.e.*, 300 mg. out of the total of 330 mg. per day put out by a 75 gm. nephrotic rat). At later stages of the disease the albumin output may be only 65 per cent of the total plasma proteins lost in urine.

2. In the fulminantly nephrotic rat, the glomerular filtering membrane presumably becomes completely permeable to plasma albumin, and concomitantly the steady state level of albumin in the plasma may approach zero.[9] Hence, if albumin input into the plasma is continuing, this protein does not stay there.

3. Of greater metabolic cogency than the amount of plasma albumin lost from the body in terms of percentage of the daily protein intake is that the quantity of 300 mg. of excreted albumin is 3 times greater than the so-called daily replacement rate of albumin in the total blood plasma of a normal rat of 75 gm. body weight.[9, 11, 12] Albumin production actually is greatly stepped up. Therefore, in the severely nephrotic rat, a unique opportunity was afforded to measure unequivocally—through its spillage in urine—the functional potential or metabolic capacity of the organism (and of the liver, in charge of this biosynthetic activity) to produce plasma albumin. The conclusion seems inescapable that in the fulminantly nephrotic rat the rate of hepatic plasma albumin production is maximally accelerated, and the rate is 3-fold normal! With the exception of the gamma globulins, all the plasma proteins are constructed by the liver.[26] Despite the greater urinary spillage of plasma albumin, in nephrosis all the other plasma proteins of hepatic origin are also fabricated at rates accelerated above normal at least as much as that deduced for albumin or even more.[9, 11, 12, 15, 25] Apparently the liver plays no favorites in this game, or may the inference be made that the response of

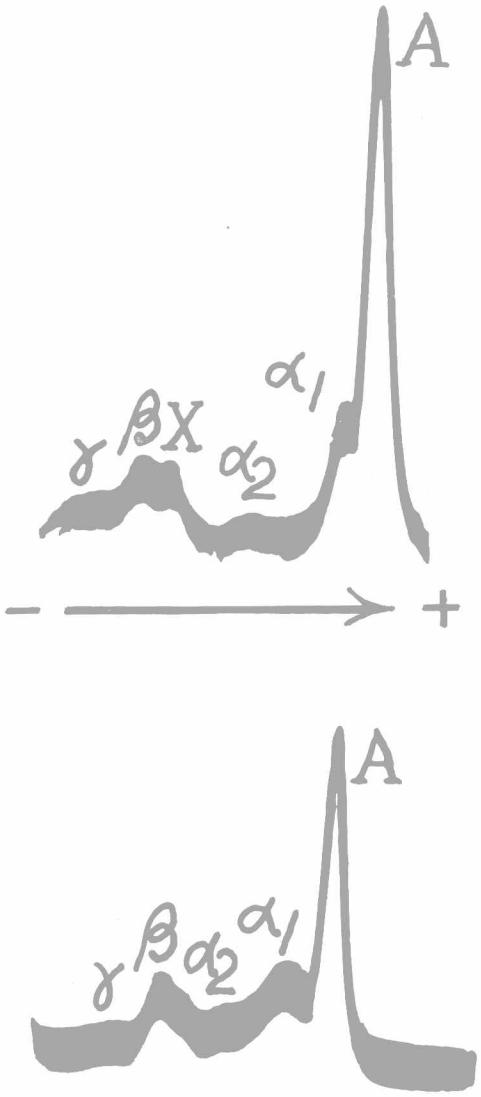

FIGURE 4. Electrophoretic patterns of normal rat plasma after 120 min. (top) and of nephrotic rat urine after 90 min. of migration (bottom). The arrow indicates the direction of migration. The symbol *A* is for albumin, α_1, α_2, X, β, and γ for the globulins. X, usually not identified in human plasma, is probably a β globulin.[9, 25] The electrophoretogram of the urine was made on the fourth day of the experimental disease, when the concentration of albumin in the plasma was very low (*cf.*, Marsh & Drabkin[12]).

heightened plasma protein production is not finely adjusted to the specific need of a particular plasma protein?

4. The urinary spillage level of the different plasma proteins is conditioned by their molecular sizes. Thus, albumin (mol. wt. = 68,000), seromucoids (mol. wt. = approximately 40,000) and siderophilin or transferrin, the plasma iron transporter (mol. wt. = 90,000), spill most readily, [9, 12, 25] whereas the high molecular weight (or low density) beta lipoproteins (mol. wt. = approximately 1,000,000) do not spill.[12]

5. In nephrosis the *steady state levels* of the proteins in the plasma are related to two main factors, the rate of inflow into the plasma and the rate of outflow into urine. Appreciably lower than normal plasma levels are associated with those proteins which escape readily from the body through the damaged glomerular filter. On the other hand, with greatly enlarged hepatic synthesis, the steady state plasma levels may be significantly increased for those proteins to which the filtering membrane still remains relatively impermeable. The large increase in the plasma levels of the lipoproteins, particularly of the beta type, is *not compensatory* in the usual sense. However, if I may anticipate, it is important in the interpretation or explanation of the mechanism of the hitherto ill-understood or mysterious hyperlipemia, which is such a constant companion of nephrosis that this disease has been given a Christian name, "lipid."

The uncontrolled loss of protein from the body by spillage into urine in severe nephrosis must be regarded as the initiating factor—whatever else may be involved—in the rapid development of a protein deficit state and the body's subsequent responses to this stressful situation. That the stress is a crucial one is evident from adrenal enlargement, which occurs early after administration of the nephrotoxic serum, and the very rapid appearance of the characteristic signs of the disease. Moreover, the fulminant form of experimental nephrosis is irreversible and quickly lethal.

The liver is the "metabolic mill" of the body and it is not astonishing that it should become so centrally involved in the metabolic responses, or attempted adjustments to a primary lesion at a distance, in the glomerular filtering apparatus. The liver is somehow driven to work at its utmost capacity to produce plasma proteins. For this hard job—doomed to failure—the liver "flexes its muscles" and hypertrophies.[10] Its greatly increased construction of plasma proteins is accompanied by appreciable increases in DNA and RNA,[10] now familiar in the synthesis of liver tissue proteins, which attends liver regeneration after partial hepatectomy.[6] May I digress or pause a moment to inquire about the liver's immense labors in so greatly accelerating its production of the plasma proteins. "Is this a good or wise thing?" "Does the liver do too good a job, and, in effect, 'overproduce' plasma proteins or 'overchannel' its metabolic resources into these particular commodities?" With reference to another of this day's