13067

Changes in Chemical Composition of the Blood Coming from Damaged Tissues.*

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Among the various theories put forward to explain the symptoms of "shock",[1] the toxic theory appears to have the largest number of supporters at the present time. It assumes that a toxic metabolite or metabolites (histamine or other protein decomposition products) are liberated from the damaged area into the blood and secondarily produce the systemic changes typical of this condition. However, it proved impossible to demonstrate the presence in the blood of toxic amounts of such metabolites during shock. Perhaps the best evidence in favor of the toxic theory is that in the case of temporary occlusion of all the blood vessels leading to an extremity, no condition of shock develops as long as the vessels remain occluded—even though the extremity is severely damaged as a result of anoxia—but release of the circulation causes rapid development of shock. This is supposedly due to intoxication of the body with toxic metabolites discharged from the damaged limb.

In a series of experiments on 6 male Macaca mulatta monkeys weighing 7 to 9 lbs, we confirmed these observations. We noted that if a rubber tourniquet is placed tightly around the upper part of one thigh for a period of 6 hours no noteworthy systemic damage is produced. The blood pressure and body temperature remained practically normal but about 15 minutes after the release of the ligature, the pulse became very feeble and the blood pressure decreased to such an extent that in most cases it was impossible to measure it. In these animals, the systemic signs of damage occurred before any significant amount of edema was demonstrable in the damaged area. This is important since according to some investigators, loss of fluid through the damaged capillaries in injured parts is the cause of systemic shock. It is remarkable that although no water was given to these monkeys, more severe edema developed in the damaged leg of each of the 3 animals which recovered than in the 3 which succumbed to shock. It is also noteworthy that the

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[1] Moon, Virgil H., *Oxford Medical Publications*, 1938.

edema was more pronounced after recovery from shock than at the peak of the shock condition. All these observations speak strongly against the theory that shock is due to loss of fluid into the damaged area but are apparently explicable on the basis of the toxic theory.

Since there is no definite direct proof in favor of the toxic theory, we performed some experiments in which the blood coming from the damaged area was directly injected into adrenalectomized mice which are particularly sensitive test objects for the detection of toxic substances. Rabbits were used as donors. In a series of 3 rabbits, the femur was disarticulated at its cranial end and all soft tissues excepting the femoral artery and vein were ligated with a thread placed around them under the skin in the region of this articulation. The femoral vein was then ligated with a separate ligature and the artery temporarily occluded by placing a protective cuff of rubber around it and ligating on the rubber. This operation was performed under ether anesthesia from which the animals were allowed to recover after the intervention. Five hours later the ligature occluding the artery was released under a second ether anesthesia while blood was taken from the femoral vein of the damaged as well as the normal leg at the same time. In order to avoid blood clotting, a few drops of heparin were injected into the artery of the damaged leg at the beginning of the experiment simultaneously with the ligature of the vein. Just before bleeding the animal, 0.5 cc of heparin was injected into the general circulation. The blood collected from the 2 legs was then comparatively assayed by injecting 0.5 cc into each of 15 adrenalectomized mice weighing 25 to 30 g. The death rate among the animals receiving blood coming from the normal extremity was approximately the same as among those injected with the blood coming from the damaged leg. Thus no special toxicity could be demonstrated even in the blood derived directly from a leg which was so severely damaged by anoxia that had its circulation been reunited with the rest of the body, severe shock would have developed.

Although these experiments are not explicable on the basis of the toxic or the dehydration theory of shock, they are not incompatible with the theory proposed by one of us[2] according to which shock may be caused by "the lack of something rather than the excess of a toxic metabolite." It is indeed quite possible that damaged tissue actively attracts certain blood components of vital importance and thus depletes the rest of the body of an important substance or substances, the deficiency of which causes shock. It will be remembered

[2] Selye, Hans, *Cyclopedia of Med.*, 1940, **15**, 15.

that our understanding of vitamin deficiencies made no progress as long as these were regarded as food intoxications while the recognition of their nature as deficiency diseases—though viewed with great skepticism at the beginning—was the key to most of what we know about vitamins today.

In order to subject the deficiency hypothesis of shock to experimental investigation, we deemed it necessary, first, to establish whether damaged tissues are really merely in a catabolic state of metabolism or whether they can actively anabolize certain substances or at least remove them from the blood, more actively than normal tissues. For this purpose we made a preliminary experiment on 4 male rats weighing 240 to 338 g in which the blood vessels of one hind leg were temporarily occluded for a period of 5 hours using the same method as described above in connection with our experiments on the rabbit. It was found that the blood sugar coming from the vein of the intact leg varied between 100-124 mg % (Hartmann, Schafer, Somogyi method) while the venous blood coming from the leg whose circulation had been occluded contained only 50-72 mg % glucose. We concluded that in certain respects at least, the metabolism of the damaged leg is more active than that of the intact extremity. It appeared possible, however, that most of the blood recovered was that which stagnated in the occluded vessels and that the blood sugar was lowered mainly because the tissues had used all the sugar originally present in this particular portion of blood. It seemed important, therefore, to see whether a damaged leg would continue to remove sugar from the circulation even if the blood flow were reëstablished for a longer time. To test this, we decided to take 2 specimens of blood, one immediately after reëstablishment of the arterial inflow and one about 15 seconds later. Since the blood escaping through the vein during this interval was discarded, it is evident that the second specimen could not have contained blood which had stagnated in the leg during the occlusion period. It also appeared of interest to determine the blood chlorides, hemoglobin, blood N.P.N. and muscle chlorides comparatively in the 2 legs so as to gain more information concerning the biochemical changes which occur in the injured area. At the same time, we wished to determine whether there is any significant difference between the biochemical changes seen after shorter and longer occlusion of the blood vessels. In order to study all these changes, we decided to use the cat which appeared more suitable than the rat for experiments necessitating larger quantities of blood. Table I summarizes our observations.

TABLE I.
Changes in the Chemical Composition of the Blood Coming from Damaged Tissues.

Experimental animal		Pregnant female cat—13 lbs			Female cat—15 lbs			Male cat—6 lbs			Female cat—10 lbs		
Blood derived from	hr	Damaged leg		Intact leg	Damaged leg		Intact leg	Damaged leg		Intact leg	Damaged leg		Intact leg
Hemoglobin	3	17.7	11.2	8.0	11.3	9.8	7.0	19.5	19.5	13.9	13	13.7	9.6
g/100 cc	6	*	*	*	9.8	8.4	7.1	15.8	16.6	11.6	15.8	14.4	7.6
Blood sugar	3	111	154	182	75	124	224	145	107	215	174	107	224
mg/100 cc	6	115	107	182	124	104	182	159	104	182	104	75	166
Blood N.P.N.†	3	75	84	58	81	71	65	75	62	58	56	62	54
mg/100 cc	6	*	*	*	72	77	56	66	64	56	64	58	56
Muscle chlorides													
mg/100 g	6	234	*	70	196	*	66	176	*	59	135	*	70

*No determination made at this time.

†It will be noted that the N.P.N. values obtained with this micro method are somewhat higher than those obtained with the macro methods. This is merely due to the different technic of preparing the protein filtrate and does not interfere with comparative determinations.

Blood samples were taken for study 3 hours after temporary occlusion of the circulation, then the circulation was occluded again and a second specimen taken 6 hours after the beginning of the experiment. In almost every case, 2 samples were taken from the damaged leg, the first immediately after reëstablishing the circulation and the second about 15 seconds later, after all the blood stagnating in the extremity had been removed. It will be seen that there is no very important difference between the chemical composition of the first and second sample nor between the determinations performed after 3 and 6 hours respectively. There is, however, a very consistent difference in the blood sugar (Hartmann, Schafer, Somogyi method) which is high in the intact leg and normal or subnormal in the damaged leg. The N.P.N. (Folin and Wu method modified for microdetermination with the photoelectric colorimeter) proved consistently higher in the damaged leg than on the other side and similarly hemoglobin (photoelectric colorimeter method) determinations indicated hemoconcentration during the passage of blood through the damaged extremity.

It is evident from these experiments that damaged tissue may utilize certain substances more actively than the corresponding normal tissues. We have no evidence that deficiency in any of the metabolites studied in this experimental series plays an essential rôle in the production of the shock syndrome. Indeed the blood chlorides rarely reach a dangerously low level in fatal shock and the systemic blood sugar is usually above normal rather than below normal in this

condition. However, there is no convincing evidence in favor of, but a good deal of observational material incompatible with, the other theories of shock while we are not aware of any observation incompatible with the deficiency theory. Our experiments indicate that increased utilization of certain metabolites (*e. g.,* sugar) can occur in the damaged area. This raises the question whether the systemic symptoms of shock might not perhaps find their explanation in that the active utilization of sugar by the damaged tissue—although unable to deplete the actual sugar reserves of the body—depletes the organism of compounds (enzyme systems, hormones, vitamins?) essential for the utilization of sugar by tissues outside of the injured area. This may perhaps also explain why the systemic blood sugar is high at a time when the blood sugar in the damaged area is low.

Summary. Experiments on monkeys show that in this primate, temporary occlusion of the blood supply to the legs does not cause any signs of shock until the circulation through the damaged leg is reëstablished. Then severe shock ensues even before any significant amount of fluid can be lost from the blood into the damaged area. This observation is incompatible with the dehydration theory of shock.

Blood taken from the vein of an extremity which was damaged by temporary occlusion of its blood supply does not prove more toxic when assayed on the adrenalectomized mouse than blood coming from an intact extremity. This observation is incompatible with the toxic theory of shock.

Experiments on the rat and cat indicate that the sugar concentration of the blood coming from a damaged extremity is significantly below the corresponding values in normal venous blood, conversely the hemoglobin and N.P.N. concentration is above normal in blood which passed through the damaged area. It appears that damaged tissues remove certain metabolites from the blood while they discharge other substances into it. These observations are considered in the light of the hypothesis according to which the general systemic signs of shock are caused by deficiency in certain substances vital for the economy of the body. According to this assumption, shock would follow after the release of temporarily occluded blood vessels not because substances coming from the limb poison the rest of the body but because active metabolic processes drain the body of certain essential compounds, the lack of which causes shock. The authors wish to point out that meanwhile they regard this interpretation merely as a working hypothesis but as such, they feel that unlike

the other shock theories, it has the advantage of being compatible with all experimental observations which have so far been made on the subject of shock.

13068

Muscular Dystrophy in the Absence of Testicular Degeneration in Vitamin E Deficiency.*

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As a result of the symptoms leading to its discovery, vitamin E has been defined as a fat-soluble essential preventing a characteristic and specific sterility in the male and female rat. Evans and Burr[1] described paralysis in the nursing young of female rats which had received minimal amounts of vitamin E. Olcott,[2] and later others, observed lesions of the skeletal muscles in the deficient young. However, the appearance of young rats weaned from stock females to a vitamin E-deficient diet is normal except for a relatively slight (compared to most deficiency states) impairment in the rate of growth until they develop paralysis of the rear limbs after many months on the diet. These paralyzed adult animals, according to Einarson and Ringsted,[3] exhibit first microscopic lesions in the central nervous system and later lesions in the voluntary muscles. Recently Knowlton, *et al.*,[4] have reported that in the long period elapsing between the appearance of gross neuro-muscular symptoms in suckling and adult E-deficient rats, chemical and functional changes and occasional necrotic fibers can be detected in the skeletal muscles in the absence of overt symptoms. Nevertheless, for many months of the rat's life, sterility remains the most prominent symptom produced thus far by a dietary deficiency of vitamin E.

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1 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

2 Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

3 Einarson, L., and Ringsted, A., 1938, *Effect of Chronic Vitamin E Deficiency on the Nervous System and the Skeletal Musculature in Adult Rats*, Oxford University Press.

4 Knowlton, G. C., Hines, H. M., and Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 453; 1939, **42**, 804.