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THE REACTION OF THE INTRAOCULAR PRESSURE TO OSMOTIC VARIATION IN THE BLOOD*

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I. Introduction

With the exception of the comparatively unimportant factor of the influence of external pressure acting directly on the globe of the eye (orbital musculature, etc.; Parsons[67], 1904), the great mass of experimental physiological investigation on the intraocular pressure has taken the form of its correlation with the blood pressure. It has been amply demonstrated (Henderson and Starling[39], 1904), that, under experimental conditions, that is, for sudden and marked changes, the intraocular pressure varies directly and very intimately with the arterial blood pressure, and authority has always stated (Leber[54], 1873; E. T. Collins[15], 1925) that proof of a controlling mechanism, other than vaso-motor, is wanting. Allowing for the fact that conditions in the ocular circulation may be widely different from those obtaining in the general systemic circulation, it would appear that the pressure in the eye may vary clinically quite independently of that in the retinal artery (Duverger and Barré[20], 1920; Velter[93], 1920; Bleidung[10], 1924, etc.). Allowing further for the consideration

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that, if the intraocular pressure varies with any element of the blood pressure, it will be with the capillary pressure—a totally different variant from arterial pressure; that, counteracting especially slow and prolonged changes, the conditions of elimination of fluid from the normal eye constitute, within limits, a very efficient mechanism to retain normality; and that, with the methods at our disposal clinically, we can obtain accurate measurements neither of the pressure of the blood nor of the intraocular fluids; nevertheless the concensus of opinion of students of the subject from the clinical aspect in every country would seem to lean to the conclusion that the two do not vary in parallel, and that, while there is an association between them, that association would seem to be accidental rather than essential. Between the physiologist and the clinician a certain divorce of opinion has thus arisen; but as all clinical phenomena must ultimately be explicable on a physiological basis, the fact that experiment is not in accord with clinical experience merely shows that other factors must be taken into account. It is probable that these concern the more subtle considerations of physical chemistry biologically applied. Neglecting for the moment the partial impermeability of the lining cells of the eye, it is generally conceded that the energy necessary for the maintenance of the intraocular pressure is derived from the blood-stream; but, in considering this, in addition to the external dynamic energy of the blood in mass movement, which is measured as blood pressure, we must also take into account the internal kinetic energy of the blood in molecular movement, which is measured as osmotic pressure.

The considerable disturbance which osmotic derangement can cause in the ocular mechanism has already been discussed in the case of diabetes (Duke-Elder[19], 1925), but this aspect of the subject has attracted a comparatively small amount of investigation. Troncoso[91] (1901), by the intravenous injection of large quantities of normal saline produced a large but transitory rise in the intraocular pressure—a rise which one would presume to correspond with a parallel rise in blood pressure. Since, however, he did not measure the latter, his results are of little value.

By the injection of hypotonic solutions, Ruben[74] (1914) and Weekers[96] (1924), produced a definite rise in intraocular pressure. Conversely, by the injection of hypertonic salt, Cantonnet[11] (1904) demonstrated a shrinkage in the volume of the eye; Scalinci[76] (1907) called attention to changes in the physico-chemical properties of the ocular fluids; Angelucci[1] (1913) using Leber's filtration manometer, concluded that the filtration rate of the aqueous was diminished, and Tristaino[90] (1912), using concentrated solutions of calcium chloride, produced definite and graduated decrements in the tension of the eye. Starting from the consideration of diabetic coma, Hertel[41] (1913) evolved a theory on an osmotic basis to account for the clinical occurrence of the soft eye met with in this condition, and later (1914)[43] he published a series of experiments wherein, working on rabbits, by the intravenous injection of anisotonic solutions of widely different substances in differing concentrations, he succeeded in producing definite variations in the ocular tension. In these experiments, however, he was content to use a Schiötz tonometer to register intraocular pressure at long intervals, and parallel records of the blood pressure were not taken; their physiological value is therefore small.

Considerably later, Magitot[60] (1923), experimenting on cats and dogs, failed to obtain any definite variation in intraocular pressure apart from the blood pressure by anisotonic injections. On increasing the strength of injection a large number of his animals died; his paper, however, makes no mention of the amount of salt used nor of the relative rate at which it was introduced. In the rabbit he obtained results comparable to those of Hertel, of which, however, he was unable to take graphic records. This discrepancy in results with different animals led him to consider Hertel's osmotic explanation improbable, and he regarded the response of the rabbit as exceptional, and, in accordance with his well-known views, dependent upon a vaso-motor effect governing the calibre of the vessels of the choroidal tunic.

In view, however, of the difficulty in accounting for the inactivity of a known and very definite physical force; in view of the great variety of the substances used by Hertel for injection (sodium chloride, sulphate, phosphate, carbonate, acetate, butyrate, isovalerianate, sugar, urea, etc.), and of the fact that their action varied, not with their chemical nature, but with their relative osmotic potential activity; in view of the fact that a similar variation has been shown by Weed[95] and his co-workers (1919) to occur in the cerebro-spinal fluid of cats and dogs; and in view of the fact that a corresponding tension variation can be induced clinically in the human eye, it would seem unlikely that the phenomenon was a peculiarity depending on the vaso-motor mechanism of the eye of the rabbit. Apart from the importance of the theoretical considerations involved and considering the clinical value of the lowering of ocular tension which can undoubtedly be obtained thus in cases of glaucoma, it would seem important that further investigation be undertaken into the question.

II. Experimental Technique

The animals used throughout the investigation were cats. Anaesthesia was induced by open ether followed by the intravenous injection of a solution of chloralose (0.075 gms. per kilo body wt. in 10 c.c. Ringer's solution). This was found to be a most satisfactory anaesthetic, and gave a uniform and perfect narcosis. It ensured the absolute immobility necessary for the undisturbed maintenance of a manometer in the eye, and under its influence the animals, with little or no attention, kept alive and in good condition without fluctuations in the blood pressure, heart and respiratory rate, and intraocular pressure (as seen in controls) over periods of observation lasting several hours. For most physiological work on pressure variations in the eye curare is indispensable to eliminate the action of the extraocular musculature. In this series of experiments, inasmuch as the change is a slow and gradual one, it was considered that this factor could be neglected. During anaesthesia the animals' body-heat was maintained by electric bulbs placed underneath the cat-table.

The intraocular pressure was recorded by a compensatory manometer of 1 mm. bore of the type introduced by von Schultén[77] (1884) and modified by Parsons[67] (1903) and Henderson and Starling[39] (1904), the compensatory mechanism being a reservoir controlled by a pulley. Saline was used as giving more accurate readings than mercury with its large inertia and its small range of variation. The constancy of the intraocular contents was assured by the introduction of a horizontal, graduated tube of capillary bore between the manometer and the eye, into which a bubble of air was admitted, any movement of which towards or away from the eye indicates entrance or exit of fluid, which must be counteracted by altering the pressure in the system by adjustment of the reservoir. The needle employed was a sharp hollow instrument, as devised by Starling, with three openings—one terminally and two laterally—to ensure free communication with the fluids of the eye, and connection by a lateral arm with the manometer left its distal end closed by a plug which could be replaced by a stylet for cleaning out any obstruction. The needle was introduced into the anterior chamber through the cornea near the limbus, parallel to the plane of the iris. On the insertion of the needle there is always some pressure reaction, but provided it is sharp enough, and pushed evenly and rapidly into the anterior chamber, this is found to be transitory. Less pressure and consequent disturbance are involved if the cornea is first partially penetrated with the point of a very sharp knife. The temporary nature of the initial disturbance was verified by the insertion of a second needle of similar bore in an experiment, and the observation of its effect on the pressure as registered by the manometer attached to the first (*cf.* Hill[44], 1913), always provided that in its introduction the iris be not injured, a complication which causes a considerable rise of tension (*cf.* Magitot[61], 1922; Weekers[96], 1922). Complete equilibrium was always obtained within 15 mins. and until such equilibrium had been attained for a control period of at least 10 mins. no experimental procedures were commenced. During the experiments, if an excursion of the air-bubble in the capillary tube of at least two degrees did not coincide with the arterial and respiratory rhythm, thus demonstrating free communication with the intraocular fluids, the experiment was discontinued. Immediately on the insertion of the needle it was carefully supported, since any traction by its weight on the cornea increases the tension of the eye and vitiates pressure records. Readings were taken continuously during the initial period of rapid variation; later, when slow pressure changes were taking place, they were recorded every three to five minutes.

The arterial pressure was recorded by the usual mercurial manometer recording on a kymographic drum, the cannula being inserted into the femoral or external iliac artery, this being the most convenient vessel of sufficient size leaving the entire circulation of the head in a normal condition. At the stage in the experiments when rapid variations occurred a continuous tracing was taken; thereafter readings were taken at 5 to 10 min. intervals to obviate difficulties due to clotting.

The venous systemic pressure was recorded by a cannula inserted proximally into the femoral vein of the side opposite to that used for arterial records, close to the crucial anastomosis, so as to obtain virtually a lateral pressure. This was connected to a vertical glass tube of 1 mm. bore into which was introduced a small quantity of physiological citrate solution. The pressure was taken by reading the resultant level in the manometer tube directly as mm. of saline. The very small amount of fluid thus entering the circulation has no appreciable effect on the general pressures (*cf.* Weed and Hughson[95], 1921). It is held by some (Becht[8], 1920) that the systemic venous pressure is a consideration of no value, but although its level at different situations is profoundly modified by local conditions, yet, to a large extent these different values vary correspondingly, and the results obtained, although in no way claiming great accuracy, would yet appear to be of some interest.

The urinary excretion was estimated by introducing a cannula into the apex of the bladder, obliterating the volume of the latter as much as possible, and counting the drops per minute flowing from it, the urethra being ligated. Here again no claim is made to great accuracy, but a sufficient degree is obtained to indicate the periods of diuresis.

In this series of experiments no attempt was made to measure directly the osmotic variation of the blood. In the absence of a more rapid or trustworthy method, recourse was had to the determination of the lowering of the freezing point, and a correlation of the results so obtained with a study of the changes occurring simultaneously in volume. It is not contended that by cryoscopy, or by a determination of any of the colligative properties of a fluid so far removed from the "perfect" solutions of van't Hoff as is blood, an exact estimation of the osmotic pressure can be made, but it is held that results can be readily obtained thus of sufficient approximation to be of value. The freezing point was estimated with the usual Beckmann apparatus, centrifugalized serum being used as being more easily manipulated; this introduces no error, since the presence of corpuscles, like other suspended particles, leaves the osmotic pressure unchanged, the plasma and corpuscles being two phases of a system in equilibrium (Hamburger[34], 1897). The blood volume was estimated, also indirectly, by a red-cell count (by the Thoma-Zeiss haemocytometer) correlated with a haemoglobin estimation (by Haldane's method). Since the total number of red cells does not change under the conditions of the experiments (haemolysis was never observed), and since the volume is altered by a fluid lacking in haemoglobin, while these values remain constant absolutely, they will change relatively to the volume, varying inversely to it.*

The solutions used for injection were given intravenously through a cannula fixed proximally into the saphenous vein, fed from a burette. By this method of administration the amount was readily measured, and the rate at which it was introduced was accurately and uniformly controlled at a time in the experiment when attention was urgently required for the observation of rapid pressure changes. This question of rate of administration was found to be very important.

The results obtained in typical experiments are represented below. A considerable amount of variation was encountered in the reaction of different animals, both as regards the degree of change, the time of incidence, and the duration, but in every case the general change showed the same constant tendency.

III. Controls

(*a*) In order to test the efficiency and constancy of the anaesthetic, the maintenance of body temperature and general condition, and the adequacy of experimental conditions throughout the periods necessitated by the nature of the observations, controls were conducted over similar intervals of time, wherein the animals were subjected to no further manipulation than the operative procedures required for the adjustment of the various recording apparatus. The example recorded (Fig. 1) shows the degree of

* The principle of this method assumes the even distribution of the red cells throughout the entire circulation, an assumption not universally granted: See Lamson [53] (1915), Krogh [52] (1920); contrast, however, Scott [78] (1917).

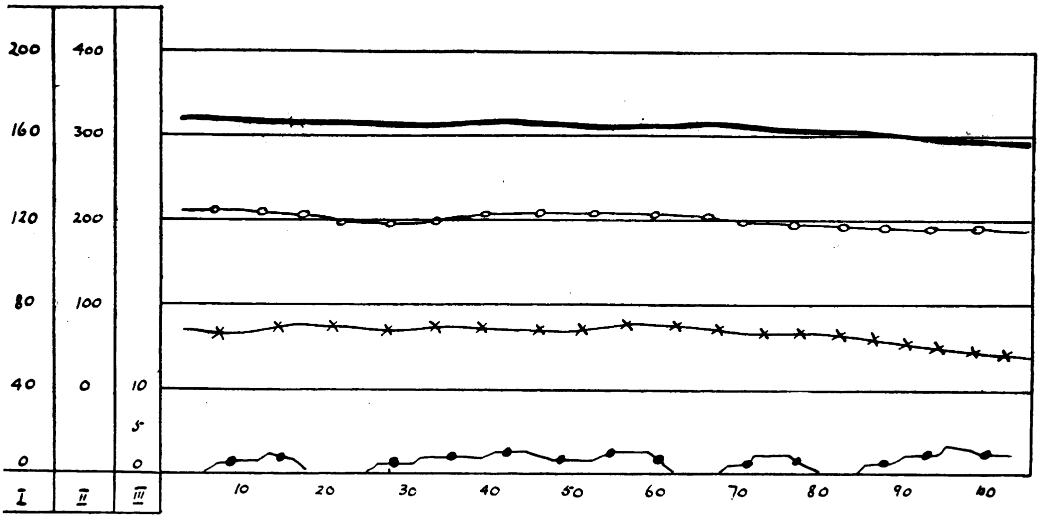

FIG. 1.

Control exper. Cat: female: 2,800 gms.: chloralose. The ordinates represent pressures: column I, arterial pressure in mm. Hg. (graphed in circled line) and venous pressure in mm. saline on same scale (graphed in crossed line); Column II, intraocular pressure in mm. saline (graphed in heavy line); Column III, urine in drops per min. (graphed in dotted line). The abscissae represent time in mins.

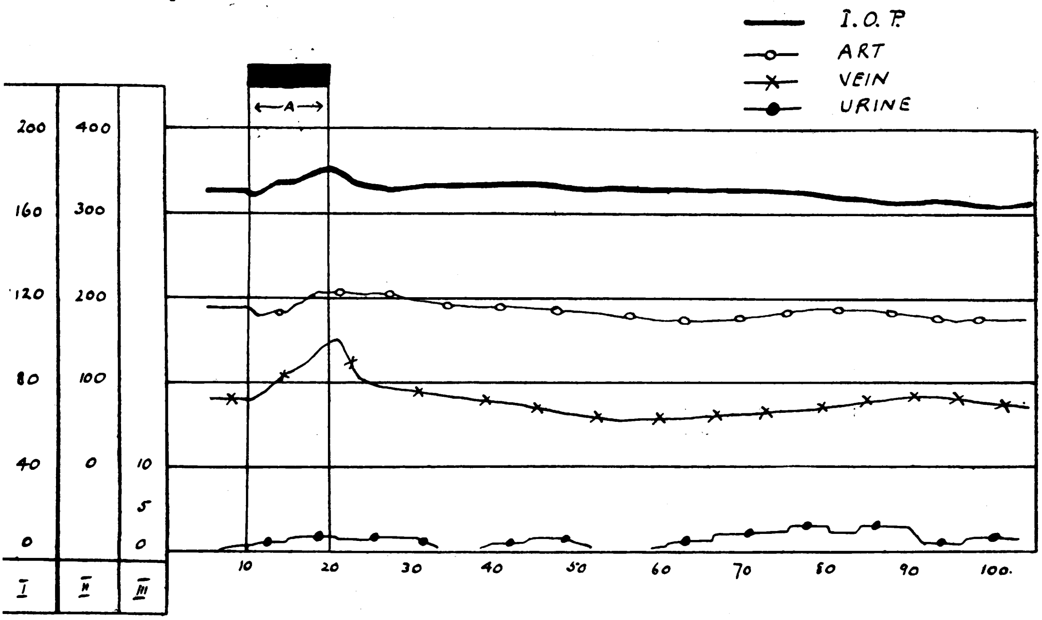

FIG. 2.

Control exper. Injection of normal saline. Cat: female: 3,200 gms.: chloralose. The ordinates represent pressures as in Fig. 1; the abscissae time in mins. During interval A 30 c.c., 0.9 per cent. saline injected intravenously.

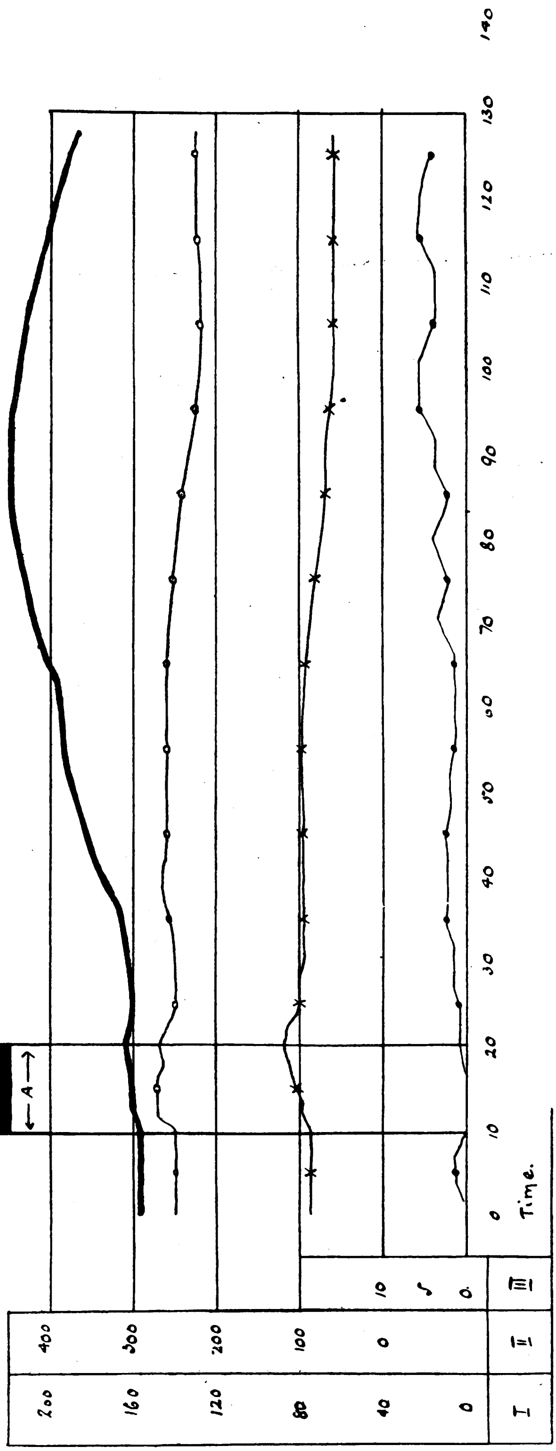

FIG. 3.

Hypotonic injection. Cat: female: 2,800 gms.: chloralose. The ordinates represent pressures, read and graphed as in Fig. 1; the abscissae time in mins. During interval A, 25 c.c., 0.3 per cent. saline injected intravenously.

constancy obtained, and demonstrates that the conditions of experimentation had eliminated error and were such as to obtain an adequate result. On the basis of Fig. 1 all the other charts should be interpreted.

(*b*) Isotonic Injections. Since the general effect of any injection will be due partly to the effect on the blood pressure of the injection mass itself, it is essential to eliminate this influence in order to assess accurately the effect due to any additional osmotic influence. Experiments were therefore done (Fig. 2) to show this purely volumetric effect, in which isotonic (0.9 per cent.) saline was introduced in amounts equivalent to the quantities of anisotonic solution used subsequently. The arterial pressure is seen to fall slightly, and then to rise somewhat above its initial level; thereafter it fell very gradually until, 20 mins. after the end of the injection, it had reached its previous level, below which it sank slightly, and subsequently rose again. The venous pressure during the injections rose markedly, and thereafter fell as quickly, remaining slightly below its initial level for almost an hour. The urine excretion was not greatly affected; if anything it was increased. The intraocular pressure showed a close and intimate connection with the blood pressure throughout; a slight initial fall followed by a rise during the injection, thereafter a decrease followed by a rise in level.

The effect therefore of an isotonic injection is very small; it is purely mechanical and varies directly with the amount injected; and the intraocular pressure follows closely the curve of the blood pressure. The general vascular effect—an initial rise, soon becoming subnormal—is well recognized. Experimental observations amply confirming this include those of Dastre and Loye(17) (1888), Cohnstein and Zuntz(14) (1888), Johannsson and Tigerstedt(49) (1889), Grosglik(29) (1890), R. Tigerstedt(89) (1907), C. Tigerstedt(88) (1908), Selig(79) (1910), Bayliss(5) (1916), Weed(95), etc. (1919), while the phenomenon is well recognized clinically in the failure of saline injections to maintain the blood pressure owing to the rapid compensation for increased volume by tissue oedema and increased excretion. The corresponding temporary rise of intraocular pressure would seem to explain the results of Troncoso(91) (1901) and to confirm the findings of Hertel(43) (1914).

IV. Anisotonic Injections—(a) Hypotonic Injections

In this series of experiments an 0.3 per cent. saline solution was used. The protocol of a typical experiment shows the following variation (Fig. 3).

The arterial pressure during the injection rose initially, fell slightly, and finally rose again through 8 mm. Hg. Five mins.

after injection it had fallen to its original height, slowly to rise again to a maximum of +6 mm. Hg. in 20 mins., after which it fell gradually. The venous pressure showed a comparatively small rise of 15 mm. saline during the injection, fell gradually, reaching its initial level in about 50 mins., and continued slowly to decrease. The intraocular pressure during the injection rose with the blood pressure, and immediately afterwards showed a tendency to decrease with it, which, however, was soon replaced by a rise which became much steeper 15 mins. after the injection, and attained a maximum of 450 mm. of saline in 75 mins., whereafter it began to fall. The urine, which remained normal at first, increased largely at about 75 mins. after the injection, the increase in diuresis in the second hour corresponding roughly with the fall in arterial and venous pressures below their initial level, and with the commencement of decline in the intraocular pressure. Before the experiment started the depression of the freezing point of the serum (Δ) was -0.628°C.; 15 mins. after the injection it had decreased to -0.613°C.; 60 mins. after it had begun to return to normal, standing at -0.618°C. No appreciable volume change could be made out.

The intraocular pressure therefore shows a variation initially parallel to both the arterial and venous curves, being of the same direction and magnitude as these; later, however, it becomes completely divorced from them, showing a marked and sustained rise over a considerable period of time.

(b) Hypertonic Injections

(1) *Hypertonic Saline.* In this series of experiments a 30 per cent. solution of sodium chloride was used; a typical variation is as follows (Fig. 4):

Immediately on injection the arterial pressure fell suddenly and rapidly from 115 to 75 mm. Hg. during the first two minutes, and then commenced to rise slowly. By the end of the injection it had risen to 90 mm.; 10 mins. afterwards it had reached its initial value; 30 mins. afterwards it had exceeded this, reaching a maximum of 130 mm.; thereafter it slowly fell to the region of its initial level. The venous pressure rose rapidly during the injection until it had almost doubled its original value (83 to 150 mm. saline). Immediately after the injection was completed it fell as rapidly during the first 5 mins., and thereafter more slowly, until, half an hour later, it had reached its normal level, after which it tended slightly to rise again. The intraocular pressure during the injection fell at first rapidly and profoundly (345 mm. saline to 180); thereupon it rose as rapidly until at the end of the injection it had exceeded its initial value and had reached a peak of 415 mm.

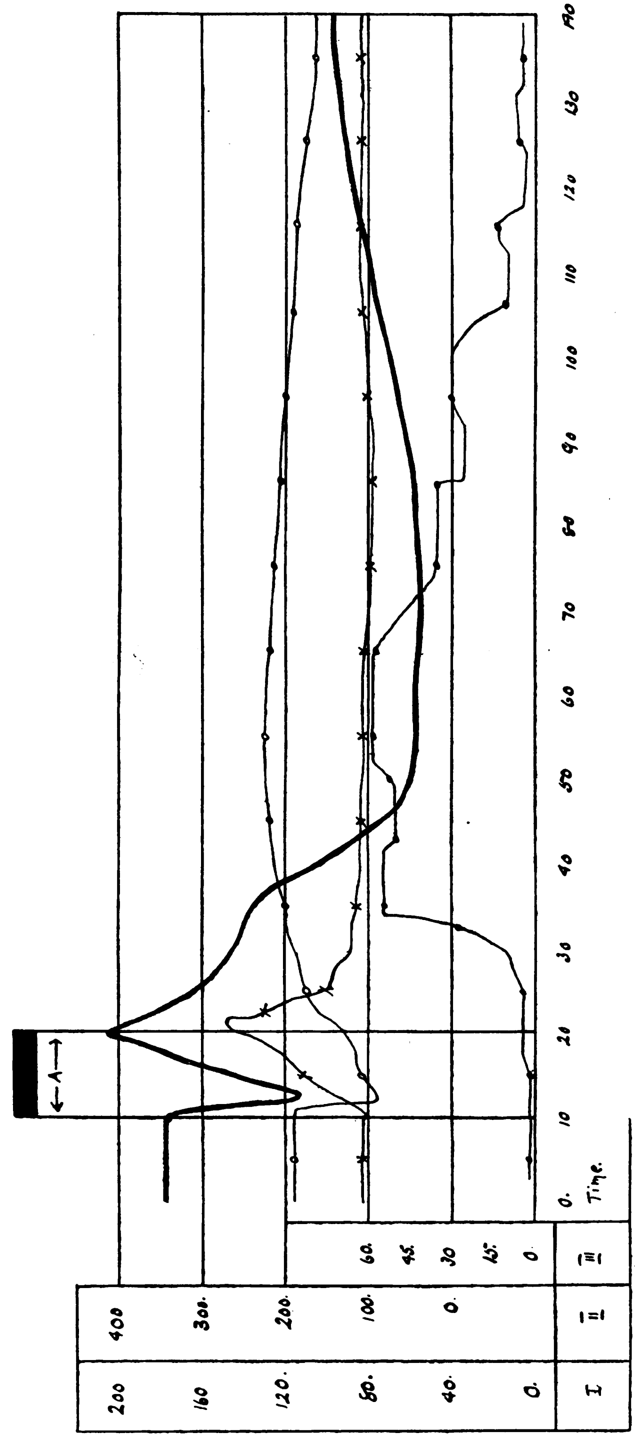

Fig. 4.

Hypertonic injection. Cat: female: 3,600 gms.: chloralose. The ordinates represent pressures read and graphed as in Fig. 1; the abscissae, time in mins. During interval A, 15 c.c., 30 per cent. saline injected intravenously.

saline. After the injection it fell rapidly, until in 30 mins. it had attained the level of 50 mm., about which figure it remained for about 40 mins., when it began to rise again. The urine excretion rose enormously about 10 minutes after the injection and remained high for about 45 mins., and thereafter gradually decreased. Before the experiment commenced the value of (Δ) was -0.629°C.; 20 mins. after the injection it had increased to -0.673°C.; 75 mins. later it had to a large extent returned to normal -0.641°C. The red count and haemoglobin index, on estimation 30 mins. after the completion of injection, were found to be 70 per cent. of their normal value, *i.e.*, the blood volume had been increased by (approximately) 30 per cent.

Between these curves a great diversity is at once evident. During the injection interval the arterial and venous pressures varied in opposite directions; for some time after, both were temporarily increased; but, looked at generally, if the transitory changes during injection be disregarded, it is seen that both remained not far removed from normal. The curve of intraocular pressure may be divided into two parts; first, during and immediately after the injection, its form may be resolved into a composition of effects of the arterial and venous pressures, the initial fall corresponding with the arterial fall of pressure, and the subsequent rise with the dominating influence of the venous pressure—a mechanical phenomenon dependent upon volume. The profound and prolonged fall which then occurs cannot in anyway be correlated with the vascular pressures, differing from them both in direction, in magnitude, and in duration. The curve of urinary excretion is again interesting in that its rise corresponds with the fall of intraocular pressure, and its gradual diminution is synchronous with the return of the latter towards the normal level.

The forms of these curves receive corroboration from the work of previous investigators. Heinz[38] (1890), the first to observe blood pressure changes on hypertonic injections, noted an initial fall until the animal died, a result confirmed by Münzer[66] (1898). Selig[79] (1910), Retzlaff[71] (1915), and Seppä[80] (1918) established that after the initial fall a subsequent rise occurred which was greater than that following the injection of isotonic solutions, a result confirmed by Bayliss[6] (1919) who showed clinically the transitory effects of such procedures in maintaining a raised blood pressure. The increase of urinary excretion has been studied by Starling[85] (1899) and Hogan[45] (1915), and the early return to normal physical properties in the blood, first suggested by the specific gravity determinations of Sherrington and Copeman[81] (1893), was confirmed by Koeppe[50] (1896) and Hamburger[34] (1902), and dealt with by Bayliss[4] (1919). The fall in intra-

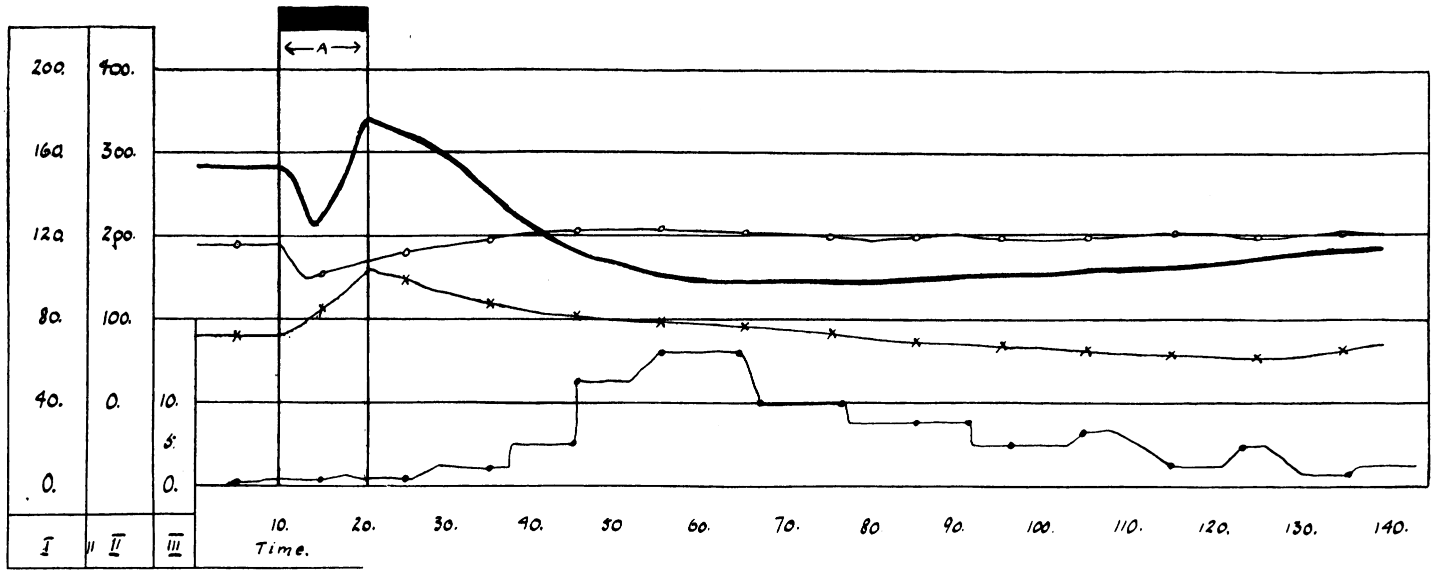

FIG. 5.

Hypertonic injection : glucose. Cat : female : 3,500 gms. Ordinates represent pressures read and graphed as in Fig. 1 ; abscissae represent time in mins. During interval A, 30 c.c. 50 per cent. glucose injected intravenously.

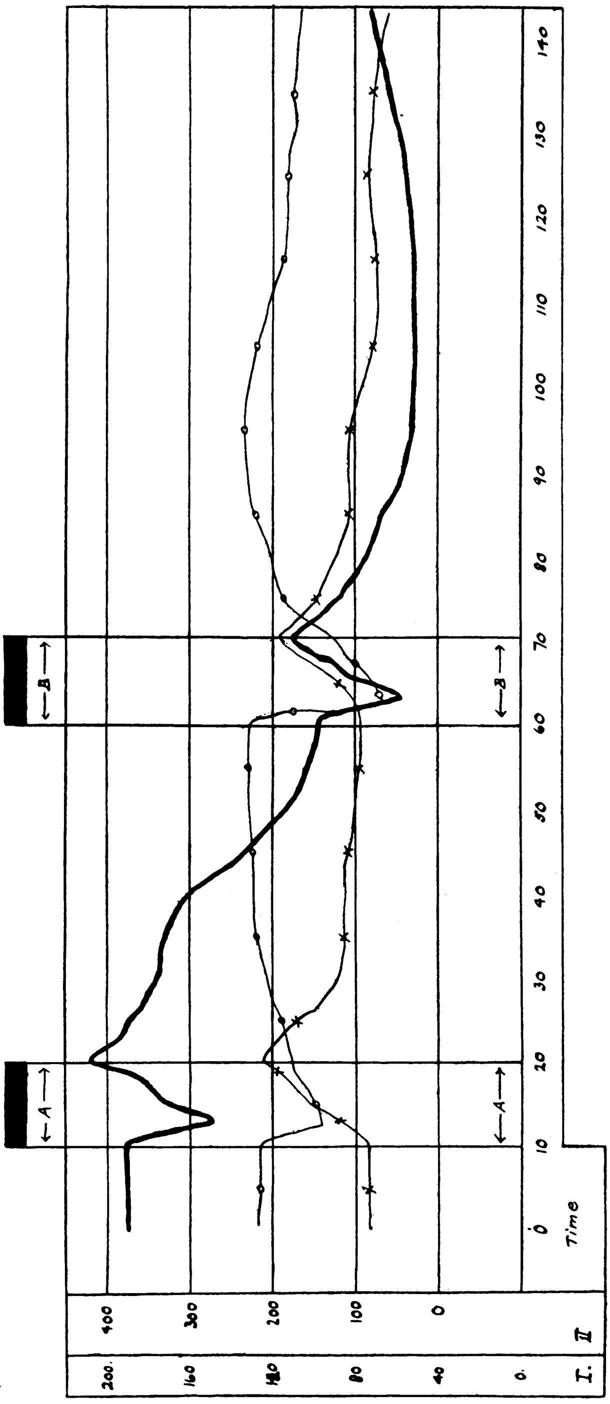

FIG. 6.

Repeated hypertonic injection. Cat: female: 3,400 gms.: chloralose. Ordinates represent pressures read and graphed as in Fig. 1; abscissae represent time in mins. During intervals A and B, 12 c.c., 30 per cent. saline injected intravenously.

ocular pressure confirms the observations of Tristaino[90] (1912) and Hertel[43] (1914), and bears a considerable resemblance to the results obtained by Weed[95] and his co-workers on the cerebro-spinal fluid, on whose technique these experiments were largely based.

(2) *Hypertonic Sugar.* If the effects noted are due to osmotic considerations, it is evident that similar results should be obtained with any substance capable of varying the osmotic pressure. A similar experiment was therefore conducted with a solution of glucose, and it showed a change of the same character (Fig. 5). Here, however, as was to be expected, the variations were not so great in magnitude, since, owing to its larger molecule and lack of ionization, the osmotic activity of a solution of sugar is very much inferior to one of salt. Thus a 30 per cent. solution of sugar is osmotically equivalent to a 4.5 per cent. solution of sodium chloride. The clinical analogue of this experiment is the occurrence of soft eye in diabetic coma.

(3) *Effect of repeated injections.* Experiments were done to determine whether the eye was refractory to repeated injections, one of which is graphically represented (Fig. 6). It is seen that a subsequent injection has an effect similar to the first, intensifying its action and prolonging the effect. In no case was it found possible to reduce the tension to *nil*, or to a negative value; before such a result was attained, the animal invariably died.

(4) *Rate of injection.* One of the most striking features in the reaction of the animal to hypertonic injections is the sudden and rapid fall of blood pressure initially, and in any clinical application of this method of reducing the tension of the eye, this factor is of great importance and necessitates considerable caution. This preliminary fall denotes a profound systemic disturbance, and it is of importance that the osmotic storm, so created by the introduction into the blood stream of a large amount of crystalloid, should be made as mild as possible. An investigation was therefore conducted into the effect of the rate of injection, typical findings of which are shown in Fig. 7. In this experiment 5 c.c. of 30 per cent. saline were given at varying rates of 20 mins., 10 mins., and 2.5 mins. The first showed a mild effect on the arterial pressure, corresponding to that obtained on the exhibition of glucose; the second showed a marked effect; and in the third the blood pressure fell very rapidly from 124 to 44 mm. of Hg., and then, after a few oscillations, dropped to 0. Meantime the animal showed a quick pulse, rapid shallow breathing, went into mild convulsions and died of cardiac and respiratory failure. The degree of disturbance entailed by the injection therefore varies as the rate of administration.

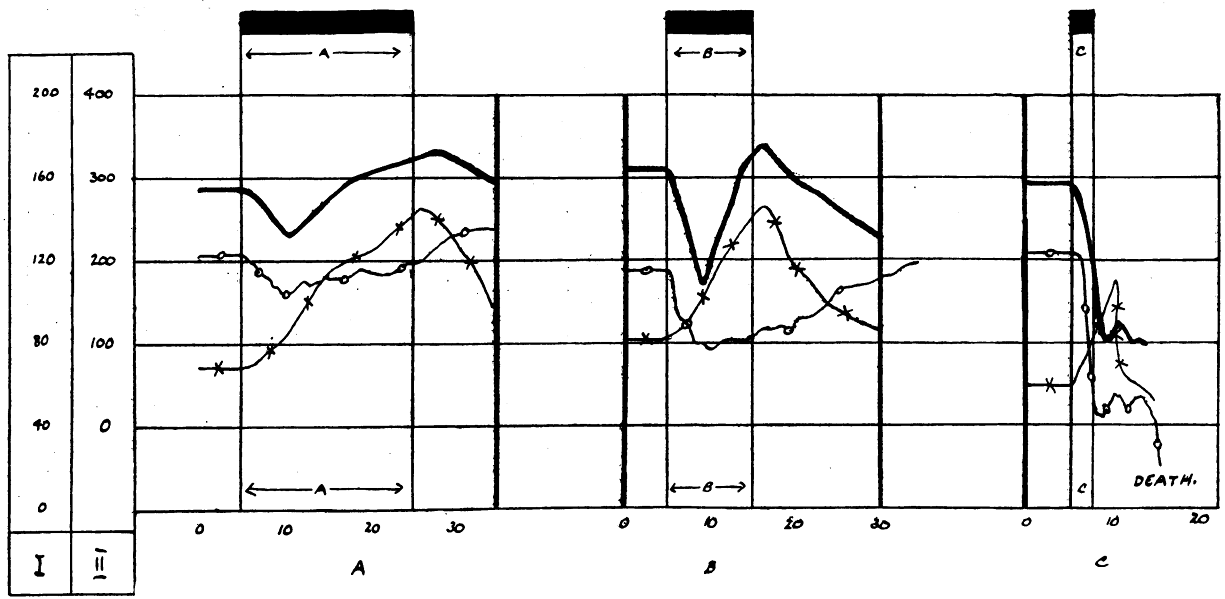

FIG. 7.

Hypertonic injections : effect of rate.
A. Cat : female : 3,700 gms. : chloralose. During interval A, 5 c.c. 30 per cent. saline intravenously.
B. Cat : female : 3,300 gms. : chloralose. During interval B, 5 c.c. 30 per cent. saline intravenously.
C. Cat : female : 2,780 gms. : chloralose. During interval C, 5 c.c. 30 per cent. saline intravenously.
Ordinates represent pressures read and graphed as in Fig. 1 ; abscissae time in mins.

Were the injection made slowly enough, even in large quantity, the processes of metabolism and excretion would be sufficient to deal with and dispose of the excess material without allowing any great alteration in osmotic equilibrium. Thus in a research to estimate the sugar-using power of men and animals by giving prolonged, uniform injections of glucose intravenously, Woodyatt, Sansum, and Wilder[101] (1915) found that in man, in the rabbit, and in the dog, 0.9 gms. of glucose per kilo. wt. (*i.e.*, 63 gms. in a man of 70 kilos.), could thus be given per hour over long periods of time without producing any pathological phenomena; above this rate glycosuria and diuresis occurred. To effect a raising of osmotic pressure a mean must be struck between these two, whereby a sufficiently large water-traffic is set up to obtain the desired result. It is obvious, therefore, that we must take as our standard of dosage, not a weight of salt, but a relative velocity-weight, *i.e.*, a weight of salt per unit of body weight, introduced per unit of time.

On these considerations depends the lethal dose of hypertonic salt, which has been very variously estimated by different investigators. Thus Guttmann[31] (1865) in rabbits, found it to be 5 gms. NaCl given as a 20 per cent. solution, Silbermann[84] (1889) states that 4 to 12 gms. kills rabbits, Heinz[38] (1890) puts it at 20 c.c. of a "concentrated" solution, Münzer[66] (1898), at 3.72 gms. per kilo. body wt., and Seppä[80] (1918) at 1.95 gms. per kilo. of animal injected at the rate of 1 c.c. of a 26.4 per cent. solution per minute. In the experiment quoted (Fig. 7) 0.8 gms. per kilo. wt. proved fatal when injected at the rate of 2 c.c. of a 30 per cent. solution per minute, and other observations showed that anything over 1.5 gms. per kilo. wt. of the same solution could not be given at the rate of 1 c.c. per minute with impunity. In the above series of experiments the average velocity-weight of salt dosage was 1.2 gms. per kilo. wt., introduced approximately at 1 c.c. per minute, thus keeping well within the lethal dose. It is evident that in any clinical application a large margin of safety should be left.

(5) *Alimentary Administration.* The same osmotic effect ought theoretically to be obtained by the administration of hypertonic solutions by any route. Experiments were therefore conducted wherein a large quantity, 150 c.c. of 30 per cent. salt solution, was introduced into the stomach of cats by a stomach tube. A typical record is figured in Chart 8. As would be expected from the slow rate of absorption, the initial sudden changes are absent; the small oscillations on the curves corresponded to vomiting movements which were difficult to eliminate, and are therefore vaso-motor effects. Apart from these incidental variations the blood pressures kept fairly level,

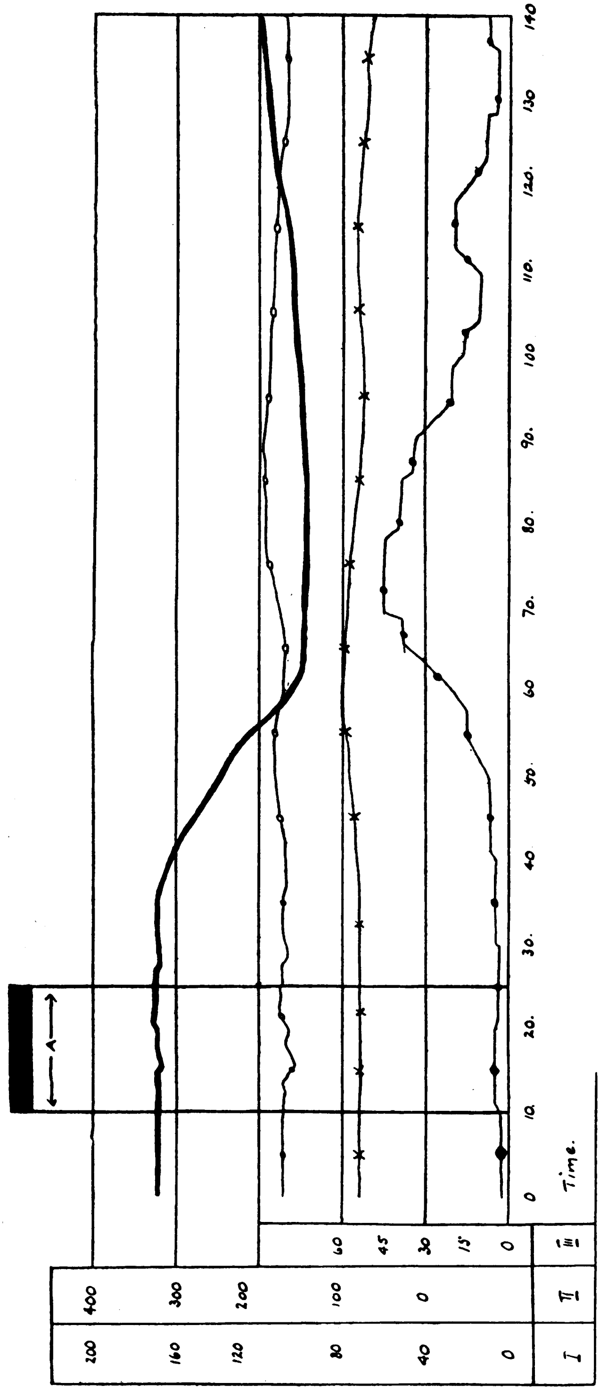

FIG. 8.

Hypertonic saline by alimentary route. Cat: female: 2,900 gms.: chloralose. Ordinates represent pressures, read and graphed as in Fig. 1; abscissae time in mins. During interval A, 130 c.c. 30 per cent. saline introduced into stomach.

showing a slight rise in both, followed by a slight fall more marked in the venous pressure than in the arterial. At first, through the initial oscillations, the curve of intraocular pressure follows closely those of the blood pressures; later it departs completely from them, falling as in the case of the intravenous injection, but to a less degree.

V. Mechanism of Action

That the reaction studied in this investigation is controlled by osmotic influences is suggested by three considerations; the nature of the change, the variety of substances causing it, and the generality of its application to different parts of the body.

A study of the nature of the pressure curves, and a comparison of the varying effect of the injection of different strengths of solution at different rates by different routes, demonstrate clearly that there are two distinct influences at work, involving two distinct responses; the first, a rapid, immediate change, following closely and depending upon the variation in part of the arterial, in larger part of the venous pressure, and therefore presumably entirely, of the (immeasurable) capillary pressure; the second, a later, more gradual, less variable change, completely independent of any blood pressure variation, differing from it in time of incidence, in rate of development, and usually in direction.

The first can be correlated with a corresponding change in the capillaries in a very striking manner. If the inner aspect of the ear of a cat with non-pigmented skin be cleaned and shaved and flooded with oil, and examined through the microscope with the aid of direct illumination derived from a brilliant light focussed by a condensing lens, a beautiful picture of the cutaneous capillary circulation can be obtained (*cf.* Lombard[(58)], 1912). On a muddy yellow background, showing up somewhat hazily, the papillae of the superficial skin vessels are seen grouped mainly round the hair-follicles as little red mounds, from which spring upwards tiny twisted capillary loops remarkably clearly defined, and between which is spread a fine network, visible clearly when the vessels nearly approach the surface, always being broken as the vessels fade away more deeply into the layers of the corium. The capillary walls themselves are not visible, but the column of blood can be readily observed, and the behaviour of the capillary bed, and its reaction to stimuli, can be studied. With the aid of a micro-scale a very exact record of degrees of change can be made. Under these conditions the average diameter of a capillary in the cat's ear is from 8 to 10 μ; the diameter of the cat's red cell is 6 μ; and under various stimuli, variations in the former ranging from 4—5 μ to 12—13 μ are easily induced. In this manner the probable conditions in the capillaries of the eye may be deduced when anomalies seem to be met with when the general blood pressure alone is measured: thus, for example, the rise of intraocular pressure coincident with the fall of blood pressure on the exhibition of amyl nitrite can be observed, by analogy, to be due to capillary dilatation. Similarly, on the injection of hypertonic salt, the capillaries of the ear are seen to constrict somewhat, and then to dilate, the dilatation gradually and very slowly subsiding, their behaviour being much as one would expect from the venous pressure and the change in blood volume, and following very closely the initial changes observed coincidently in intraocular pressure. If the ear of a rabbit be enclosed in a plethysmograph, a similar volume change, as Haupt[37] (1915) observed, can be registered. At the end of forty-five minutes, when the intraocular pressure has fallen largely, the capillaries are still dilated: the effect seems to pass off gradually and slowly, as if it were governed solely by volumetric conditions, and there would seem to be no evidence of any inherent contractile activity of the endothelial wall induced by the altered chemical constitution of the blood.

The second response can only be correlated with the physico-chemical changes occurring in the blood-stream after its flooding with dialysable substances. It is extremely unlikely that such a change could be determined by a vaso-motor influence governing the choroidal vessels as Magitot suggests. Such a change could be brought about only by either a passive dilatation or constriction of the vessels depending on volume, or by an active change determined locally by the activity of a vaso-motor nervous mechanism. The first, as we have seen, is negatived by the fact that, after a hypertonic injection, when the intraocular pressure is falling, the blood volume is increasing by 30 per cent. owing to dilution by tissue fluid, corresponding to the simultaneous rise in both arterial and venous pressures. Any passive effect on the circulation will therefore be in the sense opposite to that found in actual fact. Since a nerve impulse is determined by the passage of ions through semi-permeable membranes, it is, by its nature, susceptible to osmotic changes. That a nervous mechanism, however, cannot be the governing factor in the changes observed is evident from recent work on the effect of osmosis on nerve activity. The literature of this subject, from the time of the first observations by Eckhardt[23] (1851) and Kölliker[51] (1856) down to those of Renauld[70] (1910) and Ishikawa[48] (1912), shows much contradictory evidence. The discrepancies among these early observations arose in the adoption of the strength of a minimal (threshold) stimulus required to give a contraction response as a standard of measurement. Tanamura[87] (1916), however, showed that, in measuring osmotic pressure variations, the electrical resistance of a nerve varied with the tonicity of the immersing solution, and that such a variation of resistance caused a deviation of threshold value; thus he deduced that the threshold strength as an index of excitability implies an error due to variation of electrical resistance. Verworn[94] (1914) had previously pointed out that excitability and propagation velocity were two factors of the same physico-chemical reaction, varying parallel to each other under changing conditions. Taking advantage of the fact that the latter can be measured exactly under changing osmotic conditions, uninfluenced by any variation of electrical resistance, Shoji[82] (1919) showed that the propagation velocity, and therefore also the excitability, was at a maximum at the optimum physiological osmotic pressure, and that a higher or lower osmotic pressure acts in the same sense, decreasing the excitability and the propagation velocity in accordance with the amount of deviation from normal. Any effect of an injection of sodium chloride could only affect a nerve mechanism either chemically by virtue of the natrion itself, or physically, by osmotic change induced by the concentration of the ion. In both hypertonic and hypotonic injections the natrion is constantly present, and, moreover, a similar effect is induced by sugar; while any osmotic variation in either direction we have seen to have the same effect—decreased excitability, involving a potential vaso-dilatation with a consequent potential increase in tension; the observed changes in pressure were in opposite directions, and that induced by hypertonic solutions is of decreased tension. Their occurrence, therefore, cannot be explained on any assumptions involving a vaso-motor influence.

The variety of substances that may be employed to affect the change and their widely different chemical nature, strongly suggests that their action is merely one of physical concentration. The formidable list tried by Hertel has already been mentioned, and, moreover, this investigator demonstrated that their action can be correlated in degree with their equivalent osmotic activity.

The generality of the reaction in different parts of the body also points to the same conclusion. Mention has already been made of the closely corresponding results obtained by Weed and his co-workers in the cerebro-spinal fluid, observations which find clinical corroboration in the therapeutic lowering of intracranial pressure, in a manner exactly analogous to that shown to occur in the eye, which has been recorded in this country by Cohen[12] (1924), and MacBride and Carmichael[59] (1924), in America by Haden[32] (1919), Cushing and Foley[16] (1920), Sachs and Belcher[75] (1920), and Ebauch and Stevenson[22] (1920), and in France by Leriche[55] (1922) and Wertheimer[98] (1923). These results are all the more interesting in view of the fact that vaso-motor nerves probably do not exist in the brain (*vide* Bayliss and Hill[7], 1895).* The same method, moreover, may be employed to reduce cardiac and renal oedemas (Henriques[40], 1914).

Having established that the general reaction is an osmotic one, there remains to examine its nature in detail. It is part of the attempt on the part of the body mechanism to retain normality in the osmotic level of its fluids, a function of considerable phylogenetic age and gradual evolution. The osmotic concentration of vegetable sap appears to vary largely with the nature and habitat of the plant, as is also the case in the tissue fluids of marine invertebrates. With the development of elasmobranchs stability in level begins to be attained, which is completed first in teleosts. From these onwards, all the higher animals, including man, while they vary somewhat between species, maintain, in defiance of alteration, an osmotic pressure approximately equal to what is computed to be the value of the sea water of the early Cambrian period. How delicate is the adjustment to this level is shown by the work of Hamburger[35] (1902), whose findings are corroborated by the observations of Cohnheim[13] (1912), Ginsberg[27] (1912), Haldane and Priestley[33] (1916) and White[99] (1920). The method of adjustment can be deduced from Starling's[85] (1896) work on lymph production. In the normal state the fluid traffic of the body is regulated by the equilibrium between the amount driven out of the circulation into the tissue spaces by filtration due to the blood pressure, and that transferred back into the blood stream by the effective osmotic pressure of the blood over the lymph due to the colloids of the former. On a hypertonic injection this osmotic pressure is greatly raised by the presence of increased crystalloid. Since an effective increase over filtration pressure is thus established, fluid is transferred in excess from the tissues into the blood, dehydrating the former, and increasing the volume and pressure of the latter. As salt equilibrium is quickly re-established, the effect is only temporary, but at the same time the lessening of osmotic resistance to filtration in the kidney by dilution of the blood colloids induces a great diuresis (Barcroft and Straub[2], 1910). The very large excretion of urine observed in the above experiments, involving much tissue dehydration and corresponding with the fall of intraocular pressure, is confirmed by the experiments of Meyer and Gottlieb[64] (1914), who found that by diminishing the colloid content of the blood, urinary excretion can be obtained at a pressure as low as 13 mm. Hg. It

* It should be noted that this finding is not unquestionably accepted by everyone. For anatomical evidence of the presence of nerves, see Gulland [30] (1897), Hunter [47] (1902); for physiological evidence (action of drugs), see Wiggers [100] (1907).

also explains the rationale of still another therapeutic field in which hypertonic injections have been applied, although the physico-chemical principles underlying have not always been appreciated —the establishment of urinary flow in oligurias and anurias (Fleig[25], 1907; Henriques[40], 1914; Turrettini[92], 1915).

Converse considerations are applicable to the effect of hypotonic injections. Thus on a purely osmotic basis may be explained all the systemic effects observed in the series of experiments.

The changes in intraocular pressure are explained partially by the osmotic inflow or outflow of fluid, the eye sharing the processes of dehydration or water-logging common to all the tissues. Hertel[43] (1914) was able to demonstrate this variation in water content by enucleating one eye prior to an injection, and enucleating the other subsequently, the weight of water in each being determined by weighing the eyes and their dried residue. In each case he found that the increased tension induced by a hypotonic injection was accompanied by an increased water-content, and that the decreased tension induced by a hypertonic injection was accompanied by a decreased water content. We have been able to show that the same mechanism obtains *in vitro*. The enucleated eye has a tension of 8 to 10 mm. of mercury (*cf.* Magitot[62], 1923) which it retains for some considerable time, and only loses gradually on the disintegration of the lining cells (12 to 15 hours). The same limit of pressure is obtained on killing an animal by exsanguination (Leber[54], 1903; Grünhagen[28], 1866; v. Schultén[77] 1884). Since it is found under these conditions it cannot be due to the blood pressure, and since it remains in the enucleated eye it cannot be due to any hypothetical secretory activity; it must be dependent on the relative physiological impermeability of the integral lining cells. If such an eye be placed in isotonic saline or serum it retains its internal pressure for some time and its weight remains constant. If it be placed in hypotonic saline it increases in tension and gains weight initially, while in hypertonic solutions it rapidly becomes of pulpy consistency and loses weight. It is thus clear that the eye is capable of functioning as an osmotic machine.

The intimate nature of the mechanism is, however, probably more complicated. Owing to its ready diffusibility, the salt concentration will soon be equalized on both sides of the cellular membrane and equilibrium will be attained. The fairly prolonged effect observed clinically and experimentally, outlasting the blood changes as detected by cryoscopy, may depend on one of, or all of, the three following factors: a change in rate of elimination of fluid, a change in rate of formation—or the establishment of a new ratio between these two—and a change in the amount of fluid retained.

A disturbance of the first is suggested by the fact that with hypertonic injections Angelucci[1] (1913) noted a diminution in rate of excretion by Leber's filtration manometer.

The second factor may be studied and the two compared, by the injection of dye-stuffs. An estimation was made, by experiment, of the circulation time in the cat by injecting methylene blue into the saphenous vein and watching, ophthalmoscopically, for its occurrence in the retinal vessels; an average time interval for its appearance was from 3 to 4 seconds. Uranin was then injected and the time of its appearance in the eye as fluorescein noted, the time when the entire eye showed the fluorescein tint, and the time when clear aqueous again became apparent. In the normal eye the dye appeared clearly in from 1.25 to 1.5 minutes, it had reached its maximum density in from 20 to 22 minutes, and had begun to clear up, as demonstrated by the appearance of a negative Ehrlich's line, in 45 to 50 minutes. After a hypertonic injection its appearance was delayed to 2 to 2.5 minutes, and the commencement of its disappearance to about 70 minutes. After hypotonic injections results so consistent were not obtained; as a rule they approached very nearly the normal. It would thus seem by this rough index of measurement that with the former, at any rate, the rate of both formation and elimination is retarded.

The factor of retention of fluid is probably more important, but, involving as it does a consideration of the capillary chemistry of the colloids of the eye, its nature is so complicated that it must be left to a future discussion.*

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* A partial indication of its probable mechanism may be deduced from some considerations involving the vitreous gel. The osmotic activity of a colloid at a given concentration and temperature varies with the nature and amount of crystalloid present, independently of the osmotic effect of the crystalloid *per se*. See Lillie[56] (1907), who found that in the presence of N/96 NaCl (a much lower concentration than we are concerned with), the osmotic pressure of albumen fell from 18.0 mm. to 6.8 mm. Hg. Also Roaf[72] (1907). The process may involve an increased aggregation of colloidal particles, the first stage of that which ultimately leads to complete precipitation (see Hardy[36] 1900; Freundlich[26] 1903). Again, where a system of colloidal ions and electrolytes are involved, forces of electrostatic potential must be considered. Compare the lowering of osmotic pressure with electrolytes found by Bayliss[4] (1909), explicable by a Donnan's[18] equilibrium (1911). Compare Pauli's[68] observations (1914) on the effect of salts on the state of aggregation of proteins interpreted in terms of adsorption equilibria. Compare also the influence of hydrogen ion concentration on colloid equilibria (Michaelis,[65] 1914), its relation to the addition of salts (Loeb,[57] 1922), and its co-relation to variations in intraocular pressure (Meesman,[63] 1924; Bauermann,[8] 1924). Fischer's[24] theory (1908) of acidosis and imbibition ("Quellung"), sometime fallen into disrepute, but periodically resuscitated, is clinically and physiologically untenable. The dramatic effects he obtained *in vitro* in the eye depend; not on imbibition (solid solution, capillary adsorption), but probably on the osmotic effect of ionizing salts of proteins; see Roaf[73] (1910), Siebeck[83] (1912), Bentner[9] (1913), Procter[69] (1914); and any such effect is diminished by the addition of salts, the electrolyte diminishing ionization.

VI. Conclusions

In their classical research on intraocular pressure, Henderson and Starling[39] (1906) concluded that "the production of intraocular fluid is strictly proportional to the difference in pressure between the blood in the capillaries of the eyeball and the intraocular fluid." In the light of the present investigation this finding must be modified, since it is seen that the pressure in the eye, while influenced by the pressure in the blood stream, can vary quite independently of it.

(1) The intraocular pressure is maintained and varied by three factors (neglecting the influence of external pressure):

(*a*) The physiological partial impermeability of the lining cells of the eye.

(*b*) The hydrostatic blood pressure in the capillaries of the ocular circulation.

(*c*) The osmotic pressure of the blood and of the ocular contents.

The relative potential importance of the osmotic over the hydrostatic component is seen in that the intensity factor of the former is of the order of 5,000 mm. Hg., or about forty times that of the physiological blood pressure.

(2) The physiological partial impermeability of the endothelial cells maintains normally a pressure in the intraocular fluids of 10 mm. of mercury. This pressure component is maintained without regard to changes in the hydrostatic pressure of the blood, but it may be overcome by osmotic changes therein.

The blood pressure in the capillaries is a function of the general systemic blood pressure and the local state of vaso-dilatation. The former is to be regarded as a composition of both arterial and venous conditions; of these two, since the arterial pressure is largely damped by the interposed arterio-motor mechanism, the venous pressure is normally the preponderating. Changes in the local state of dilatation are mediated in part by a nervous mechanism acting on the Rouget cells, in part by physical and chemical influences, locally determined, acting on the contractile elements of the capillary wall. Under the influence of changes in this pressure component, the intraocular pressure may vary to a maximum not higher than that of the capillary level, less the effective difference in osmotic pressure between the blood and the aqueous due to their unequal colloid content, and to a minimum not lower than 10 mm. of mercury.

The factor of osmotic pressure acts partially in the alteration of the rate of production and elimination of the intraocular fluid, partly by the alteration of the equilibria of the physico-chemical activities involved in the dynamics of colloidal solutions. Under the influence of changes in this pressure component, the intra-ocular pressure may be either raised or lowered above or below the effective limits of variation determined by the blood pressure.

(3) Experimentally, the influence of the first factor is seen in the pressure decrement in the excised eye. The co-relation of the intraocular pressure with the blood pressure has long been recognized in the laboratory. Pressure variations caused by osmotic influences have now been demonstrated.

The alteration of intraocular pressure induced by the intravenous injection of anisotonic solutions is due, initially to changes in the hydrostatic capillary pressure, ultimately to changes in osmotic conditions.

(4) Pathologically a lowering of intraocular tension due to a diminution or abolition of the partial impermeability of the lining cells of the eye is seen as a result of the solution of the continuity of the endothelial layer that occurs physiologically in long-continued moribund states (for example, the hypotony of typhus) or that occurs anatomically after trauma, induced either accidentally, or therapeutically in a trephining operation, the filtering nature of whose scar depends on the failure in the formation of an endothelial lining on its inner surface.

The part played by the general blood pressure in the explanation of pathological tension states has been tried and found wanting: that played by the capillary pressure is still virtually an unknown quantity. The physiology of the capillary vaso-motor mechanism is still in its infancy, but recent investigations (Krogh[(52)]) on its response to mechanical and chemical stimuli, to changes in the osmotic and hydrogen ion concentration of the surrounding media, and to the action of the hormones elaborated by the endocrine glands, are full of significance.

The action of osmotic pressure in the production of pathological hypotony is seen in the soft eye of diabetic coma. The part played in the production of pathological hypertension by osmotic pressure and the many physico-chemical relations it controls has as yet been comparatively unexplored. As all normal bodily processes rest ultimately on their reaction, so must we look to their biological application for a rational explanation of those departures from normal which we recognize as disease, and the therapeutics of perfection becomes the re-establishment of equilibrium in terms of chemistry and of physics. The two major diseases of the eye —cataract and glaucoma—are patent examples of physico-chemical derangement. The latter cannot be ultimately explained on an anatomical basis, and to treat it as if it were, by the modern filtration operations can only be considered an expedient. In this connection the opinion of Sir John Parsons expressed in the Bowman Lecture (1925) is apposite: further advances in ophthalmology will not be along the lines of improvement or elaboration of operative technique, but rather by a fuller understanding of the biochemistry of the eye and of the physiology of vision.

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