26. On some Results of Ligaturing the Anterior Abdominal Vein in the Indian Toad (*Bufo stomaticus* Lütken).

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(Text-figure 1.)

Preliminary Statement.

Most of the lower Vertebrata differ from the Mammalia in that the liver has conveyed to it a quantity of venous blood which has traversed the tissues of the legs and pelvic region, in addition to the venous blood which, as in Mammals, is derived from the gut-walls and contains the digested food products. This fact that in the lower Vertebrata the liver receives a portion of ordinary non-gut venous blood has not received the attention which its possible significance deserves. So far as I know*, only one author has ever offered an explanation, and this solely appertained to the coccygeo-mesenteric vein of Birds. Owen in 1841† made the following observations : " The venous system of the kidneys is so arranged in birds that the blood can be distributed either to the portal system by the mesenteric vein [*i.e.* the blood brought to the kidneys by the femoral veins can flow *posteriorly* through the substance of the kidneys in the so-called hypogastric veins and so enter the coccygeo-mesenteric vein], or to the pulmonary system by the vena cava and right side of the heart, according to the degree of rapidity with which the pulmonary or portal systems of veins are respectively supplied, or in other words, according to the activity with which the circulation in each of these systems may be going on at two different periods . . . . This disposition has been erroneously supposed to indicate that the urine was secreted from the venous blood in birds, as in reptiles and fishes; but the end attained by the venous anastomoses in question bears a much closer relation to the peculiar necessities and habit of life of the bird, and, so far as I know, has not hitherto been explained. There is no class of animals in which there may be, at any two brief and consecutive periods of existence, a greater difference in the degree of energy and rapidity with which the respiratory functions are performed than in birds. When the bird of prey, for example, stimulated by a hungry and an empty stomach, soars aloft and sweeps the air in quest of food, the muscular energies are then strained to the utmost, the heart beats with the most forcible and rapid

* The author has not had access to literature in India.

† "On the Anatomy of the Southern Apteryx," Trans. Zool. Soc. London, vol. ii. 1841.

contractions to propel the current of blood along the systemic arteries, and the pulmonary vessels require the greatest possible supply of blood to serve the heart with the due quantity of arterialized fluid: the digestive system, on the other hand, is in a state of repose, and we may conceive the portal circulation to be at its lowest ebb. Suppose the Eagle to be glutted with his quarry and reduced to a state of torpor; the animal functions are now at rest, but the organic powers concerned in the assimilation of the food are in full play, and the portal or hepatic circulation is as active as was the pulmonary a short time before." And Owen further adds that "the anastomosis of the pelvic veins, in being the means of conveying common venous blood into the liver, goes to prove that the blood of the venæ portæ does not require any peculiar preparation by circulation in the spleen or other viscera to fit it for the secretion of bile." This explanation seems plausible, especially when we reflect that the common assumption made in nearly all modern text-books to the effect that the blood always flows *anteriorly* in the so-called hypogastric veins (also sometimes called the "renal portal" veins) of the bird is almost certainly wrong, it being, on the contrary, more than probable that the blood in these veins always flows *posteriorly* *, as conjectured long ago by Jacobson (1817), Jourdain †, and other authors. But this explanation evidently does not apply to animals like Amphibia and Reptilia, which are notoriously sluggish and yet pour into their livers a much greater proportion of non-gut venous blood than birds. Also in Mammals, which most resemble Birds in the alternating activity of the respiratory and portal systems, a communication between the post-renal and portal veins does not exist.

Now a supply of ordinary non-gut venous blood to the liver may signify (1) that the venous blood is to enable the liver to obtain a greater supply of water than it would otherwise receive, or (2) that the liver is, in part, in these forms, an organ of excretion and supplementary to the kidneys. The first supposition naturally occurs to one when Amphibia are in question, since a toad or frog certainly never drinks water by mouth, but always absorbs it by the belly and thigh skin, and this water presumably is taken to the liver by the anterior abdominal vein (and to the kidneys by the renal afferent veins), and this is probably the case in all Amphibia. Though in Fishes, Reptilia, and Birds there is no certain evidence of cutaneous absorption of water, yet it is well known that Snakes and Lizards frequently evince a desire to lie in water, and Fishes, Crocodiles, and Turtles of course habitually live in it. It may also be remarked that the anterior abdominal vein, or its equivalent, usually has factors from

* This is a subject I hope to investigate in the near future. It is almost certain that these so-called hypogastric veins of birds are the homologues of the "pelvic" veins of Amphibia and Reptiles and not of the "renal portal" veins, and if this be so, the blood must flow *posteriorly* in them.

† M. S. Jourdain, "Recherches sur la Veine Porte Renale." Annales des Sciences Naturelles, 4 ser., Zoologie, Tome xii, 1859, p. 134.

the walls of the urinary bladder, which, after all, is a possible source of water. Finally, it is noteworthy that, for some reason or other, Reptiles and Birds (even when aquatic) conserve their water very carefully, as we may realize when we note the semi-solid character of their urine*, the absence of sweat and mammary glands and their non-fœtal rearing of offspring; whereas Mammals, on the other hand, are very prodigal with water in all these respects. Hence it is possible that, comparatively little water being taken into the gut, the liver "arranges for" an accessory supply of blood in order to satisfy its water requirements.

As to the liver, regarded as an organ of excretion in lower Vertebrata, I have no evidence.

That in the Common Frog, however (and therefore in all other animals with anterior abdominal veins), the anterior abdominal vein supply to the liver is not essential to the life of the animal is proved by the abnormalities occasionally found†, in which the anterior abdominal opens into a pre-caval instead of into a hepatic-portal vein, the animal apparently being normal in all other respects.

The Ligaturing of the Anterior Abdominal Vein in the Common Indian Toad.

While in India in 1915 and 1916, I determined to ascertain the effects of ligaturing the anterior abdominal vein, though it is evident that this operation will not give results comparable with those to be found in the abnormalities just referred to, because in the latter the blood-flow in other veins is not interfered with, while in a toad with a ligatured anterior abdominal vein the blood, which would otherwise flow through this, is forced to pass along the two renal afferent veins—the result being increased blood-pressure in the renal afferent veins and interference with the renal arterial circulation‡.

In all I performed thirty-four experiments. The method I finally adopted was to anæsthetize the toad with ether, cut through the belly skin and the underlying muscular body-wall for two or three centimetres, ligature the anterior abdominal vein in two places and remove the portion of the vein in between. Both the body-wall and the skin were sewn up with sterilized silk thread

* Sharpe's statement (Amer. Jour. Physiology, vol. xxxi.) that the water of the urine of birds is absorbed in the rectum, a thick paste of uric acid remaining, is in all probability incorrect—the semi-solid urine of birds and reptiles is found in this form in the ureters, and is so secreted by the kidneys. See Appendix in my paper "On the 'Renal Portal' System (Renal Venous Meshwork) and Kidney Excretion in Vertebrata," shortly to be published.

† Woodland, W. N. F., Zool. Anzeiger, Bd. xxxv. 1910, p. 626. Also O'Donoghue, C. H., *ibid.* Bd. xxxvii. 1911, p. 36.

‡ See my paper "On the 'Renal Portal' System (Renal Venous Meshwork) and Kidney Excretion in Vertebrata," Parts I. and II., shortly to be published. The results of double perfusion and other experiments prove that as the pressure in the renal afferent veins increases, the arterial flow becomes retarded, and above a certain point is stopped altogether. Ligaturing the anterior abdominal vein in the Indian toad more than doubles the amount of blood in each renal afferent vein.

and the wound cleansed with weak carbolic acid, carbolic ointment being rubbed on the surface. While the wound was healing, I covered it with a pad of cotton wool, protected with a waterproof sheet, the four corners of which were drawn out and tied anteriorly above the scapulæ and under the animal's throat, and posteriorly over its back. The animals were given an occasional bath in shallow distilled water (the skin having previously been well cleaned), but were normally kept dry, the pad protecting the wound from urine and fæcal matter in the basin.

Out of 11 toads operated on as above described (save that I only ligatured the anterior abdominal vein in one place) in 1915, one lived for ten days, one for fourteen days, one a day short of eight weeks, and two others were *killed* after eight weeks and eleven weeks respectively. I shall only record my examination of the three long-period survivors, all of which were operated on on October 1st, 1915, and the wounds healed by October 12th. A day or so later they were all active and feeding well.

One, as already stated, died on November 26th, one day short of eight weeks after the operation. It was very thin, and had been ill during the previous week. It weighed at death (after subtracting weight of food in gut) 21·5 gms. The heart was normal in size (heart ratio = 229·0 *) ; the liver a trifle large (liver ratio = 21·5 *). The liver was very diseased, being full of small cysts; the spleen was much enlarged and also full of cysts, and the fat-bodies very minute. Sex not recorded. The anterior abdominal vein was found to be well ligatured and was quite empty ; on the other hand, the renal afferent veins and post-caval were very large, and the two kidneys (quite healthy) were gorged with venous blood and therefore rather dark in colour. It is important to note that *a new anterior abdominal vein had not been formed*. The kidneys, after as usual being slightly squeezed and all attached vessels removed, weighed together 0·230 gm., and *were therefore apparently enormously enlarged* (kidney ratio = 93·5 *, body-weight taken at death).

The toad (a male) which I killed on November 28th—eight weeks and one day after the operation—was perfectly healthy, being active and feeding well, and all the internal organs in perfect condition. In this toad I found to my surprise that *a new vein had been formed posterior to the ligature and entering the liver, also two or three small new veins coming from the muscles of the anterior ventral body-wall and opening into the principal new vein* (text-fig. 1, B) †. The body-weight (after weight of food in gut subtracted) was 21·9 gms. The heart ratio was 199·3 * ; the liver (weighing exactly 1·0 gm.) ratio = 23·9 * ; the kidney ratio = 164·8 *. The kidneys, therefore, were about normal in size.

The remaining toad (a male) I killed on December 18th—eleven weeks after the operation—and this also was in perfect health inside and out. In this toad also *a new vein had been formed* to allow the ligatured anterior abdominal to become functional. The body-weight was 30·0 gms. The heart ratio = 229·0 * ; the liver ratio = 19·6 * ; the kidney ratio = 157·9 *. The kidneys, therefore, were about normal in size.

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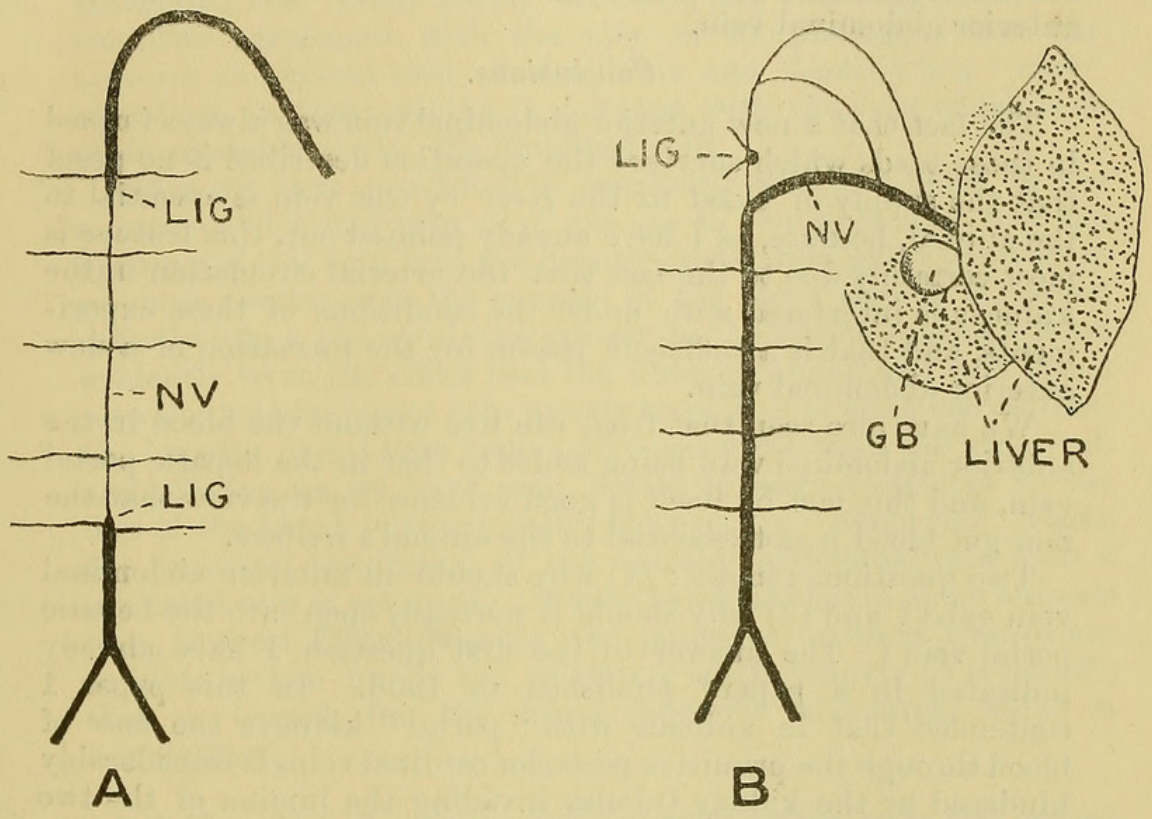
* The normal (average) ratio— $\frac{\text{Wt. of body}}{\text{Wt. of viscus}}$ —for the heart (all vessels cut off and all blood washed out and dried) in these toads is 216·8 (determined in 29 toads) ; the normal ratio for the liver (squeezed and dried) was 28·4 (18 toads) ; the normal ratio for the kidneys (wt. of both kidneys, attached ureters and vessels being removed) was 237·5 (82 toads). I must add, however, that in two other lots of toads the average kidney ratios were 155·2 (10 toads) and 159·3 (6 toads). The kidney ratio (also the heart and liver ratios) shows great variations, not connected with the time of year or with sex.

† Compare the " rapid formation of a collateral circulation so that the blood could get round the ligature to the liver " (Starling, ' Principles of Human Physiology,' 1912, p. 858) when in a mammal the portal vein was ligatured off from the liver and made to open into the posterior vena cava. In this case, however, the liver is deprived of blood from the gut and not merely of an additional supply of ordinary venous blood, as in the toads with ligatured renal afferent veins.

In 1916 I was unfortunate in this experiment, possibly owing to my predilection for giving the animals a bath every morning, but probably also owing to the fact that in each operation I ligatured the anterior abdominal vein in two places and cut out a portion about half a centimetre in length in between. Out of 23 toads operated on only five survived for more than eleven days. That this was due largely to the mode of operation and not solely to the ligature of the anterior abdominal vein is shown

Text-figure 1.



A. Small regenerated anterior abdominal vein in a toad which was killed four weeks after the operation. LIG, positions of the two ligatures, the intervening portion having been cut out in the operation. NV, new vein formed.

B. Large regenerated anterior abdominal vein in a toad killed eight weeks and one day after the operation, which merely consisted of the vein being ligatured in one position. GB, gall-bladder; other letters as in A.

I cannot guarantee the absolute fidelity of these figures, since they have been copied from rather rough sketches made when I dissected the toads.

by the fact that out of ten control toads in which an identical operation was performed, save that the exposed anterior abdominal vein was not ligatured, nine of them did not survive for longer than eleven days, and only one for six weeks and two days.

Of the five survivors of the actual operation in 1916, one which survived eleven days had a kidney ratio (in all these ratios, unlike those of 1915, the weight of the body is that taken at date of operation) of 119·7; another which survived twelve days had a similar kidney ratio of 134·2; another survivor of two weeks and one day had a kidney ratio of 95·8. Only one toad completely survived the operation, and this was a female which I *killed* four weeks after the operation. The animal was then in perfect health inside and out, and in this case I found that *a new vein had been formed* to enable the anterior abdominal vein to continue to function (text-fig. 1, A). The animal weighed 23·4 gms. on the date of operation (July 22nd), but when killed

on August 19th only weighed 14·6 gms. (after allowing for weight of food in gut). All internal organs were quite healthy, and the renal afferent veins were very large, due of course to the small size of the newly-formed portion of the anterior abdominal. The heart had apparently become much reduced in size, or was naturally small, the ratio being (weight of body at date of operation) 325·0; the liver was also extremely small (ratio=67·2); and *the kidneys were below the average size* (ratio=260·0).

Summarizing the results of these experiments, we may conclude that the ligature of the anterior abdominal vein caused either the death of the animal or the re-formation of the anterior abdominal vein: in no case did an animal survive in a healthy condition for a considerable length of time without a functional anterior abdominal vein.

Conclusions.

The fact that a new anterior abdominal vein was always formed in those toads which survived the operation described is no proof that the supply of blood to the liver by this vein is essential to the animal, because, as I have already pointed out, this feature is most certainly due to the fact that the arterial circulation in the kidneys is interfered with under the conditions of these experiments, and that is a sufficient reason for the formation of a new anterior abdominal vein.

We have also seen that frogs can live without the blood in the anterior abdominal vein being added to that in the hepatic portal vein, and this fact by itself is good evidence for the view that the non-gut blood is not essential to the animal's welfare.

Two questions remain: (1) why should an anterior abdominal vein exist? and (2) why should it normally open into the hepatic portal vein? The answer to the first question I have already indicated in a paper* published in 1906. In this paper I contended that in animals with "portal" kidneys the flow of blood through the primitive posterior cardinal veins is considerably hindered by the kidney tubules invading the lumina of the two veins and subdividing them up into coarse networks of sinusoids (Shore †, Minot ‡), and that the anterior abdominal vein is formed as an alternative route to relieve the congestion consequent on the formation of the "renal portal" system. Judging from recent measurements of the relative diameters of the renal afferent and anterior abdominal veins in *Bufo stomaticus* and *Rana temporaria* respectively, I find § that in the Indian toad about three-fifths of the venous blood from the legs flows to the heart via the anterior abdominal vein and about two-fifths via the two renal afferent veins, and that in the frog (*R. temporaria*) about one-half of the blood flows by each of these two routes, from which we may conclude that the resistance to flow of the blood offered by the liver capillary system is in the toad about one-third and in the frog about one-half of that offered by the renal venous meshwork ("renal portal" system) of *each* kidney. From other evidence §

* Woodland, W. N. F., Proc. Zool. Soc. London, 1906, p. 886.

† Shore, T. W., Jour. Anat. Physiology, vol. xvi. (n.s.) 1901.

‡ Minot, C. S., Proc. Boston Soc. Nat. Hist. vol. xxviii. (10) 1898, p. 265.

§ See Part II. of my paper "On the 'Renal Portal' System etc.," shortly to be published.

I have also concluded that the resistance offered to the blood traversing the hepatic portal system is very little more than that experienced by the blood when the anterior abdominal vein opens directly into a pre-caval vein, and this is supported by the fact that in such abnormalities (in which the anterior abdominal opens directly into a pre-caval) the renal afferent veins are of about the normal size. The renal venous meshwork in both kidneys then offering considerable resistance to the passage of blood from the hind limbs and tail, I again suggest that this blood has sought an additional path by means of which it can reach the heart without traversing the "renal portal" systems, and this suggestion is in complete agreement with the view of morphologists that the anterior abdominal vein of Amphibia and Reptilia is a "new" structure, and has nothing in common with the vein which it so often resembles—viz., the umbilical vein.

Since we have provisionally concluded that the blood in the anterior abdominal vein is of no use to the liver, and that the opening of this vein into the hepatic portal vein offers practically no more resistance to the passage of the blood than if it opened directly into a pre-caval, the answer to the second question must evidently be to the effect that the anterior abdominal vein opens into the hepatic portal vein merely because it is more convenient, the hepatic portal vein being more accessible than the pre-caval, and offering, as we have seen, but little more resistance to the flow of the blood. Occasionally, however (in "abnormal" frogs), the ancestral connection of the anterior abdominal vein with a pre-caval vein is retained. It may be added that in some animals (*e.g.*, in most Elasmobranchs) the posterior cardinal sinuses are so little broken up by the relatively small kidneys (Vialleton*) that a bypath for the blood (anterior abdominal vein) is unnecessary.

* Vialleton, M. L., "Caractères lymphatiques de certaines veines chez quelques Squales." C. R. Hebdom. des Séances de la Soc. Biol. Paris, Tome liv. 1902.