TABLE II. Skin Collagen Expressed as Percent Collagen Dry Fat Free Sample.

Casein	Wk 1		Wk 2		Wk 3	
	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C
8%	33.5	35.0	42.0	37.3	42.4	43.2
22%	34.0	32.9	37.2	34.6	37.7	39.4

Discussion. The results of these experiments indicate a direct action of ascorbic acid on the initial biosynthesis of collagen in implanted polyvinyl sponges in the rat. This effect was much more marked under conditions of higher protein intake. It is quite probable that the mechanism for rapid collagen formation requires ascorbic acid as a component or that the vitamin may serve to expedite earlier release of some inhibitory mechanism resulting in rapid biosynthesis of collagen. The negative results obtained on skin collagen may be explained on the basis of the extremely slow turnover of body collagen as compared to such rapid synthesis as that encountered in the experimental system employed.

Summary. Our results indicate that on a diet containing 22% casein, extra amounts of dietary ascorbic acid caused a substantial increment in collagen synthesis which was significant during the first 2 weeks after sponge implantation. It was also increased by Vit. C supplementation of diets containing 8% casein but the results were less marked than with the higher protein level. The results also indicate that addition of extra amounts of Vit. C to these diets improved their biological value from the point of view of biosynthesis of collagen but not for growth.

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Countercurrent Exchange in Vessels of Renal Medulla. (26462)

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After the proposal of the countercurrent multiplier hypothesis of renal medullary function, Wirz(1) was able to show by micropuncture experiments that the papillary blood supply was subject to renal countercurrent osmotic concentration and dilution. Berliner *et al.*(2) in restating and modifying the countercurrent hypothesis considered the medullary circulation to be simply a passive countercurrent exchange system working in parallel with an active tubular countercurrent system. Gottschalk and Mylle(3) have concurred in this view, and presented data confirming Wirz's original observations.

Where a gradient for any diffusible material exists between its 2 ends, a countercurrent exchanger acts as a barrier to the net transport of that material along its long axis. The more diffusible the material, the more effective the barrier. In a passive system concentrations of sodium, chloride, and urea, the principal elements of the papillary hy-

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perosmolarity of mammalian kidney, would tend to be conserved more effectively than concentrations of large molecules such as the plasma proteins, but less effectively than gases or other lipid-soluble blood components. Although such an arrangement poses difficulties in terms of gaseous exchange between the deep medulla and the general circulation, the results of the following experiments on incorporation into the papilla of the rat of materials of varying diffusibility are consistent with this simple hypothesis.

Methods. Male Sprague-Dawley rats weighing 200-250 g were used. The animals were kept on normal laboratory diet and had spontaneously hypertonic urine. Incorporation into the renal papilla of radioactive tracer substances of widely different diffusibilities was studied. In some animals only one tracer was employed, in other series 2 tracers were employed simultaneously.

Incorporation of a highly diffusible substance into renal papilla. Radioactive Kr^{85} was used in a concentration of about 50 μc/ml air. The unanesthetized rats were confined in a small glass container with carbon dioxide-absorbing pellets. By injecting oxygen, negative pressure and asphyxia were prevented. At varying time intervals (1,3,6,10, 60 and 90 minutes) the animals were dropped into liquid nitrogen. The frozen animals were sliced transversely on a band saw and autographs prepared by placing X-ray film and the still frozen body sections together at dry ice temperatures. After development, relative Kr^{85} concentrations of the various tissues were estimated by densitometry of the films.

In all animals the cortex of the kidney had approximately the same density on the films as the blood in the heart. This was taken to indicate that the renal cortex is saturated rapidly (in less than one minute) with the inert gas. In the animals sacrificed after 90 minutes of Kr^{85} inhalation autographs of the kidney showed an average papillary density of 93% of the cortical density. This was taken to indicate that the solubility of Kr^{85} in the papilla was 0.93 of that in the cortex. On the basis of these two observations, incorporation of Kr^{85} into the papilla was established from the ratio of density of the X-ray film over the renal papilla to that over the renal cortex divided by the factor 0.93.

Incorporation of a colloidal substance into the renal papilla. A qualitative estimate of the state of albumin incorporation after 15 seconds perfusion was attempted. Nembutal anesthetized rats were injected intravenously with about 40 μc I^{131}-labeled human serum albumin. Kidneys were ligated at the end of the specified interval, removed, and frozen in dry ice. Radioautographs of unfixed frozen sections were made using ordinary X-ray film.

Simultaneous papillary incorporation of a highly diffusible substance and a colloidal substance. The mode of incorporation of 2 substances of widely different diffusion characteristics was studied in rats using the *in situ* perfusion technic described by Sellers *et al.*(4). The perfusate was about 12 cc of heparinized rat blood at about 25°C. Microcurie amounts of Na^{24} and I^{131}-iodinated human serum albumin or of I^{131}-antipyrine and Au^{198}-colloidal gold were added to the perfusate. A Nembutal anesthetized rat was prepared with one loose tie around each renal pedicle, and a loose tie around the aorta above the renal arteries. A needle of the size of the aorta was inserted into the aorta below the renal arteries. Through this needle the perfusate was introduced rapidly under a hydrostatic pressure of about 140 mm Hg. Simultaneously the aorta was tied above the renal arteries, and the inferior vena cava was cut open. In this way an *in situ* renal perfusion was established. After 10 to 15 seconds, when the supply of perfusate was almost exhausted, both kidneys were tied off, cut free, and placed on dry ice. The renal papilla, a sample of tissue from the renal cortex, and a sample of the perfusate were placed in small vials, and assayed for radioactivity using a well-type scintillation counter. After 2 counts of the samples at separate times, use of the decay factors of the isotopes involved permitted calculation of the original ratio of the two isotopes in each sample.

Results. Incorporation of a highly diffusible substance into papilla. A radioautograph obtained from a rat breathing Kr^{85}

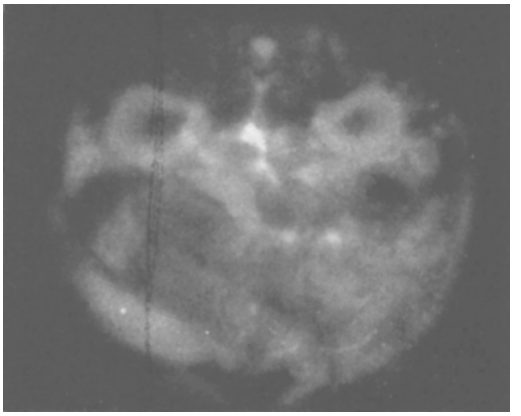

FIG. 1. Autograph, transverse section from rat breathing Kr^{85} for 3 min. Both kidneys (r. and l. at top) show low activity in papillae.

for 3 min is given in Fig. 1. The 2 kidneys are outlined clearly with the cortex standing out as a bright ring. In contrast, the deeper parts of the medulla are quite dark. Thus, relative to the cortex, the papilla is saturated only slowly with Kr^{85}. Results of the densitometer readings from all the animals sacrificed at varying times of exposure are given in Fig. 2.

Incorporation of a colloidal substance into papilla. A radioautograph obtained from a rat kidney ligated 15 seconds after intravenous injection of a small amount of I^{131}-iodinated human serum albumin is shown in Fig. 3. Even at such a short interval incorporation of albumin into papilla is greater than the corresponding incorporation into cortex. Thus incorporation of the large albumin molecule in papilla is, on gross evaluation, fast compared to that of highly diffusible Kr^{85}, as presented above. This autograph also shows that the vascular bundles of the inner medulla become distinctly outlined by 15 seconds.

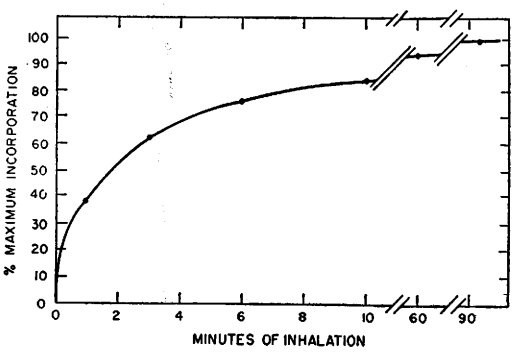

FIG. 2. Rate of incorporation of Kr^{85} into papilla of rat kidney as determined densitometrically from autographs as in Fig. 1.

Simultaneous incorporation of a highly diffusible substance and a colloidal substance. Results of aortic infusion experiments using a mixture of Na^{24} and I^{131}-albumin in 8 rats are given in Table I. The autograph of Fig. 3 indicates the distribution of I^{131}-albumin in kidneys of such animals. Autographs of sodium distribution after this interval have been illustrated by Krakusin and Jennings (5.) The quantitative results indicate that under the conditions of these experiments, of the amounts of sodium and albumin available, the cortex incorporates an average of more than 3 times as much sodium as albumin, but in the papilla only about half as much of available sodium is incorporated as of available albumin. Stated in another way, if compared with the simultaneous incorporation of albumin into the two tissues, sodium under the conditions of our experiments is incorporated into the cortex about 6 times as rapidly as it is into the papilla.

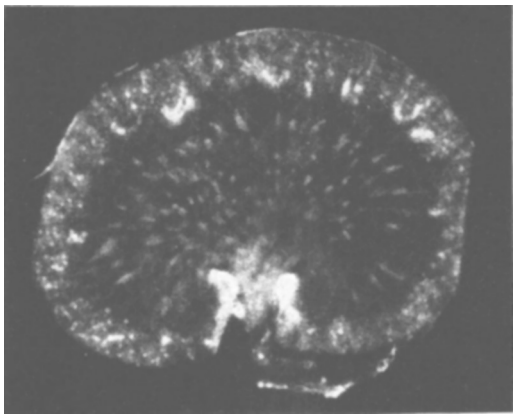

FIG. 3. Autograph, kidney of rat 15 sec. after inj. of I^{131}-human serum albumin. Note activity in papilla. Activity in central portion is in medullary vascular bundles, here cut obliquely. In cortex activity is probably due largely to intraepithelial accumulation of tracer.

Similar calculations on 4 rats perfused with a mixture of I^{131}-antipyrine and Au^{198}-colloidal gold indicate that in comparison to the simultaneous incorporation of colloidal

TABLE I. Relative Incorporation of Na^{24} and I^{131}-Albumin into Renal Cortex and Renal Papilla of Rats (Aortic Perfusion Experiments Lasting 10 to 15 Sec.).

Rat No.	Kidney	Ratio Na^{24}/I^{131} Cortex/Perfusate	Papilla/Perfusate
1	R	3.03	.24
	L	3.33	.33
2	R	2.94	.69
	L	3.45	.63
3	R	4.00	.75
	L	2.63	.60
4	R	2.63	.42
	L	3.23	.56
5	R	4.35	.42
	L	1.75	.48
6	R	2.77	.65
	L	4.00	.68
7	R	3.87	.48
	L	1.73	.21
8	R	3.74	.45
	L	3.25	.26
Mean values		3.18	.49

gold in the 2 zones, antipyrine enters the cortex 20 times as rapidly as it does the papilla. In 3 of these 4 rats on the same basis the rate of cortical incorporation exceeds papillary incorporation by more than 100 times.

Discussion. Our various experiments are not on a comparable quantitative basis, but the qualitative order of effectiveness of exclusion of the various materials studied seems established. It is reasonable to assume that both serum albumin and colloidal gold have very low rates of diffusibility, essentially zero in comparison to sodium, antipyrine, and krypton. Sodium enters the papilla less readily than albumin, but much more readily than antipyrine does in comparison with colloidal gold. Sodium is therefore excluded with intermediate effectiveness. The observations with krypton cannot be related directly to our other data, but comparing its slow entry into the papilla with the heavy concentration of Na^{22} found by Krakusin and Jennings(5) in the rat papilla within 30 seconds of injection, its exclusion must be regarded as effective compared with that of sodium. Thus the various materials studied are seen to be excluded with an order of effectiveness corresponding to the anticipated order of permeability of capillaries to each of them. This is what would be expected in a passive countercurrent exchange system. These findings are consistent with those of White *et al.*(6) that water is more effectively excluded from the renal medulla than is sodium.

This conclusion invites comparison once again between the countercurrent system in the renal medulla and the swimbladder in certain fishes. The apparent similarity in both microscopic and submicroscopic structure of the retia mirabilia involved in the 2 cases has been pointed out(7). Attention may also be drawn to the interesting but unexplained tendency of red cells to be absent in the efferent vessels in both cases(7,8). And now, in spite of the seemingly overcomplex fine structures of the retia mirabilia in the rat and toadfish for this function, the objective evidence so far indicates in both cases that only passive countercurrent exchange is involved.

The striking similarity of structure and function in the swimbladder and renal retia mirabilia is even more remarkable when one considers that the countercurrent conservation required in the 2 cases poses significantly different problems. Gases are the most diffusible of the normal molecular population of the blood by a wide margin; their equilibration in a countercurrent system will be achieved almost entirely through exchange of gas molecules, and without any other significant effect on the composition of the blood passing through the rete. On the other hand, the sodium, chloride, and urea constituting the bulk of the osmotic gradient being guarded in the renal papilla are alike in being much less readily exchangeable than gases, and furthermore are also, in other sites, considerably less exchangeable than water (9).

Important consequences follow from this. Whereas conservation of the oxygen gradient across the swimbladder rete necessitates only exchange of oxygen, the effective countercurrent conservation of sodium, chloride, and urea can only be achieved at the expense of severely embarrassed transport of gases into and out of the area of containment, and nec-

essarily involves significant movements of water as well. That this problem of gaseous exchange is a real one in the kidney is shown by our krypton autographs. The low oxygen tension observed in papillary tissue and in urine(10) is probably directly related to it. The high rate of anaerobic glycolysis found for papillary tissue(11) may well represent an adaptive response to an unavoidable and permanent oxygen lack. Speculation on this problem may lead one to ask if any part of the oxygen needs of the papilla might be met by oxygen freed *in situ,* perhaps by a mechanism such as that evidently embodied in the swimbladder gas gland; or, alternatively, whether some modification of the rete vessels in the kidneys does not occur to reduce their permeability to gases and thereby increase the amount of any gas that can be carried the length of the rete. The increase in urinary oxygen tension during water diuresis(12) appears to conflict with both these possibilities, but does not exclude them conclusively.

Given normal characteristics of capillary permeability, equilibration of opposing streams of blood unbalanced with respect to sodium, chloride, and urea will involve not only movements of solutes, but also large bulk movements of water. The direction of this movement in the kidney, as Gottschalk and Mylle(3) have stated, will be such that water will be shunted past the papilla. The expected consequence of this will be that nondiffusible components of the blood will be concentrated to levels above normal in vessels of the papilla. This conclusion has been anticipated for nondiffusible solutes from observations of the tendency of plasma protein to appear in papillary vessels of sectioned material as "colloid"(13), and from tracer determinations of apparent papillary plasma protein "volume" as greater than any likely estimate of the true vascular volume of this tissue(14). Recently Thureau (unpublished results) has shown directly as great as 3-fold concentration of protein over normal plasma levels in samples of plasma obtained by micropuncture from vessels of the hamster papilla. Protein concentration in papillary plasma from this viewpoint occurs only as an unavoidable consequence of conserving materials less exchangeable than water by the countercurrent method.

Gottschalk and Mylle(3) reached their conclusion above on theoretical grounds and from observing what they considered elevated red cell concentrations in superficial vessels of the hamster papilla. While the conclusion is evidently correct in the case of plasma proteins, it should be noted that the reported concentration of red cells is in conflict with the conspicuous absence of red cells in the papilla and its efferent vessels in histological preparations(7), the typically pallid appearance of papillary tissue, and with apparent papillary hematocrits of 5% or less obtained by tracer methods(15). A red cell shunt past the papilla even more effective than the shunt for water is demanded to fit these findings. The observation of Gottschalk and Mylle suggests that some of the superficial vessels of the papilla receive their blood from the vessels in the inner stripe (the zone immediately outside the papilla) through which the bulk of the red cells entering the arteriolae rectae must be shunted, but should not be taken as evidence of a generally elevated papillary hematocrit.

Summary. 1) The countercurrent action of the renal medullary vasculature, apparently passive, is demonstrated by its differential exclusion of materials from the papilla of the kidney in order of diffusibility. 2) The structural and functional similarity in the rete mirabilia of the kidney and fish swimbladder is stressed. The differences in functional detail in the two cases are proposed to relate to differences in the materials for which gradients are being maintained.

We are indebted to Dr. R. W. Berliner for suggesting the possible significance of a study of rate of incorporation of highly diffusible substances into the renal medulla, and for his continued interest and helpful criticism during the study. The technical assistance of Mrs. H. J. Burtner has been of the greatest value.

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Fractionation and Immunological Properties of a DNA-rich Preparation from *Brucella abortus*.*† (26463)

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Previously we described the isolation of DNA-rich preparations from *Brucella abortus* by extraction with 0.5% phenol(1), and the ability of such preparations to produce antisera containing antibodies that precipitated DNase-sensitive antigens(2,3). Subsequent studies(4) revealed that such antisera also contained antibodies to antigens insensitive to DNase treatment, and that only 5% of all immunized rabbits were capable of producing antibodies against DNase-sensitive antigens. This variability may be similar to that encountered in studies of *Lupus erythematosus*, in which antibody-like serum factors reacting with DNA were found irregularly, and to the irregularity with which antibodies to haptenes are produced. With this variability in mind, the present report is restricted to an analysis of a lot of DNA-rich material from *B. abortus*, hereafter referred to as preparation #12, which contained DNase-sensitive antigens. Preparation #12, as well as previously used preparations, consisted largely of DNA, but also contained some RNA, carbohydrate, and about 25% protein that could not be dissociated from the nucleic acid(1). An attempt was therefore made to establish more precisely the chemical basis for the immunogolical specificity by fractionating the preparation containing the antigens. Progress made in such purification and the properties of the several fractions obtained will be described.

Materials and methods. Immunizing antigens. Preparation #12 ("#12 prep") was obtained from mucoid cells of *Brucella abortus*, strain 19, according to Braun *et al.*(1). It was separated into several fractions by differential ultracentrifugation as described below. *Preparation of antisera.* New Zealand white rabbits (5-6 lb.) were immunized by intravenous injections of a saline solution of either the unfractionated preparation or its fractions. In the course of immunization, each rabbit received a total of 2.5 mg of nucleic acid, as determined by absorption at 260 mμ, or that quantity of a given fraction which was obtainable from 2.5 mg nucleic acid in the unfractionated preparation. Injections were spaced over a period of 4 weeks, with doses of unfractionated material ranging from 0.05 to 0.25 mg of nucleic acid and equivalent amounts of the fractions. The

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