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Measurement of Renal Medullary Blood Flow

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TRUETA, BARCLAY, DANIEL, FRANKLIN and PRICHARD[1] took serial X-ray photographs of rabbit kidneys after i.v. injection of thorotrast. In 1947 they wrote: '...all the evidence at our disposal indicates that in normal circumstances the circulation through the medulla is relatively slow as compared with the circulation through the cortex...' This particular finding of TRUETA and co-workers attracted relatively little notice, but another development soon provided a new impetus for the study of renal medullary circulation. In articles that laid the experimental foundation of the countercurrent hypothesis WIRZ, HARGITAY and KUHN[2] and WIRZ[3] emphasized the importance of medullary blood flow in the renal concentrating process. Subsequently, many investigators turned their attention to the morphology and the function of the renal medullary circulation. In 1964 and 1967 outstanding reviews were published by THURAU[4,5] on medullary blood flow. The present review will summarize recent work on the morphology of the medullary circulation and examine new methods [6,7] for measuring blood flow through the renal medulla.

Morphological and functional aspects

Macroscopic anatomy and histology have long justified a division of the mammalian kidney into relatively homogeneous regions such as cortex, outer and inner medulla. There is good evidence that a similar division is warranted on functional grounds as well. The renal medulla receives its blood supply through the efferent vessels of the juxtamedullary glomeruli and in addition, according to FOURMAN and MOFFAT[8], through a background capillary network which is continuous throughout the cortex and medulla. These authors concluded that the latter pathway provides an alternative supply when the main medullary vessels are constricted. ROLLHÄUSER, KRIZ, and HEINKE[9] disagreed with this view and stated that, in the rat kidney, the sole source of blood to the medulla is through the efferent vessels of juxtamedullary glomeruli and that three regions: outer and inner regions of the outer medulla and inner medulla receive relatively independent supplies from vasa recta bundles. FOURMAN and MOFFAT postulated that, in the rat, a sphincter-like constriction of the juxtamedullary efferent arterioles, possibly acting together with a general constriction of the vasa recta in the outer medulla, might reduce greatly the flow through the vascular bundles, and that this might occur in dehydration and after administration of antidiuretic hormone. They quoted evidence for a cholinergic vasomotor influence in the juxtamedullary region.

O'MORCHOE[10] carried out a study of the vascular bundles in the outer medulla of dog kidneys. He found that descending vasa in any one bundle may originate from as many as 10 efferent arterioles of juxtamedullary glomeruli. In descending towards the inner medulla the bundles divide, and occasionally side branches join the surrounding capillary plexus. Although the total number per unit area of descending and ascending vasa throughout the outer medulla is relatively constant, the average diameter of both types of vessels decreases towards the inner medulla. The area occupied by the bundles, 12–14% of the total cross section in the outer part of the outer zone, decreases to about 6% in the region adjacent to the inner medulla. It seems likely that a few large bundles would be well

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suited for countercurrent exchange between adjacent vessels within the bundles and would exchange material less effectively with the environment, whereas many smaller bundles would exchange diffusible material more actively with the surrounding tissue. The structural dissymmetry that is apparent when the bundles are viewed from cortical and inner medullary directions, raises the question whether such a structure would be equally suitable to exclude as well as to trap diffusible substances, i.e. whether it would have the properties of a valve. A schema of the renal circulatory bed as represented by O'MORCHOE, is shown in Figure 1.

A comprehensive study was made by LEVER and KRIZ[11,12] of the spatial interrelationship between vascular and tubular elements in the medulla. These authors found that in the outer zone, around the vascular bundles which contain the alternating descending and ascending vasa in a strictly geometrical arrangement, further regularities are apparent. Thus, in a ring that surrounds the bundle, descending limbs of the loop of Henle alternate regularly with ascending vasa and, to the periphery of this ring, ascending limbs of the loop of Henle are associated with collecting ducts enmeshed in a capillary plexus. In the inner medulla, although regularities are less visible, a clear pattern still emerges. Ascending thin limbs of the loops of Henle retain their association with collecting ducts and descending limbs with ascending vasa, and, as in the outer medulla, the 2 limbs of the loop of Henle are systematically separated from each other. A close relationship between loops of Henle and surrounding blood vessels was also observed by SABOUR[13] who found that a common basement membrane is frequently shared by these channels. Orderly interrelations in vascular and tubular architecture are of great importance in understanding function, and the analysis of the urine concentrating process set forth by LEVER and KRIZ is both novel and challenging.

A quantitative functional co-ordination between vascular and tubular flows was first noted by KRAMER, THURAU and DEETJEN[14] who calculated that medullary flow rates of blood and tubular fluid are of the same order of magnitude. THURAU, DEETJEN and GÜNZLER[15] concluded that an initially isolated increase in medullary blood flow is, of necessity, followed by an increase of flow in the loop of Henle. If reabsorption of water and sodium remains constant distal to the turn of the loops of Henle, increased medullary blood flow should be manifest in increased urine output and/or sodium excretion. THURAU and associates attributed the diuretic effect of rising arterial blood pressure to this mechanism. Later, the same mechanism was held responsible for the diuresis during saline loading by EARLY and FRIEDLER[16] and for the increase in sodium excretion that accompanied intraarterial infusion of acetylcholine by PINTER, O'MORCHOE and SIKAND[17]. The evidence for this mechanism remains inferential until medullary blood flow and loops of Henle flow can be measured in the same preparation.

Methodological aspets

Medullary blood flow has not as yet been measured directly, and all techniques employed so far are applications of the tracer method. Hence arises a dilemma: a fundamental requirement of this method is that the tracer should not separate from the traced substance [cf. discussions of perfect *v.* imperfect tracers and of incomplete mixing by SHEPPARD[18]]. If a tracer is used for flow measurement, at some relevant point it should be homogeneously mixed with the flowing fluid. However, the kidney is a sorting device of unprecedented versatility, effective in separating particles ranging in size from protons to red blood cells. Not only does it

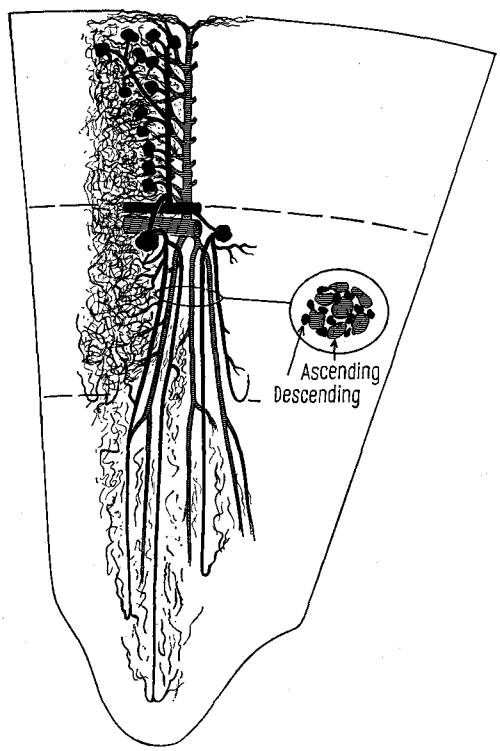

Fig. 1. Schema of the renal vascular bed.

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separate urine from blood and lymph but, in so doing, it carries out numerous intermediary separations that are not manifest in the final product. These processes affect the handling of most tracers by the renal tissue. PERL and CHINARD[19] observed that transit curves of various tracers in the renal vein show many unique characteristics, and interpreted these curves in terms of a convection-diffusion model.

Specific techniques

Photoelectric measurements in tissue. These measurements of regional blood flow in dog kidneys were carried out by KRAMER, THURAU and DEETJEN, who published their classical studies in 1960[14,20]. In 1964 in co-operation with BRECHTELSBAUER and MEIER, 2 further publications were added by the same investigators[21,22]. For medullary measurements a small light source was inserted into the tissue and a photocell was placed via ureter over the surface of the renal pyramid; cortical flow was recorded by a light reflection sensing device placed over the renal surface. Evans blue or cardiogreen dyes were injected into the renal artery and passage of the dye was recorded. Average cortical and medullary mean transit times ($\bar{t}$) were 2.5 and 27.7 sec, respectively, which corresponded to a perfusion rate per 100 g tissue of 440 ml/min in the cortex and 21.8 ml/min in the medulla. Subsequently[21] a value of 112 ml/min was calculated for the outer medulla. In denervated kidneys, of the total renal blood flow 92.5% passed through the cortex, 6.5% through the outer and 1% through the inner medulla. The medullary transit time of Evans blue was decreased by water and osmotic diuresis and by an increase in arterial blood pressure. The authors concluded that autoregulation of blood flow is not operative in the medulla.

The experimental arrangement of KRAMER and associates differs from the conventional dye dilution technique in 2 respects: (1) observations were made inside the circulatory labyrinth, and (2) elements of tracer were within view of the detector for variable lengths of time, i.e. some tracer particles were counted more than once.

KRAMER and associates assumed that the average site of observation was half way along the elementary channels and that the record could be interpreted as a tracer transit curve through one half of the circulatory labyrinth. In the cortex this assumption might not be adequate since, according to KÜLGELGEN, KUHLO, KUHLO and OTTO[23], the surface of the kidney is rich in small veins. For this reason, the average site of observation might be downstream from mid-volume. KRAMER and associates carried out several measurements in various depths in the medulla using diffuse and spot illumination through the tissue. In accordance with direct histological evidence they concluded that the ascending vasa are wider than the descending.

The second factor affects medullary measurements more than cortical since some tracer particles may take a relatively short route while others may follow contorted pathways, or may disappear and reappear in the view of the detector. Therefore, the observed curve can neither be transformed, simply by a scaling factor, to a density function of transit times, nor is the value of abscissa at the center of gravity of the curve a measure of the mean transit time of tracer through half of the vascular bed. This raises the question of what interpretation may be given to tracer transit curves in tissue. ZIERLER[24] developed a formula for calculating mean transit time from curves obtained with external monitoring: at any time the amount of tracer within view of the detector equals the difference between the quantity which has entered the region and that which has left it prior to that time. If tracer input into the observed region is instantaneous or input is complete before the tracer begins to leave, the mean transit time is obtained by dividing the area under the curve by the peak ordinate (A/P). Although these simplifying assumptions may not always hold, further investigation by ZIERLER[25] pointed to experimental designs by which good estimates of $\bar{t}$ may be obtained.

In recent papers AUKLAND and WOLGAST[26] and WOLGAST[27] reported 'mean' transit time through one half the volume of the vascular bed of the medulla as the time that divided the area under the transit curve into 2 equal parts. If the record could be interpreted as a frequency function, this value would represent the median which, like the center of gravity of the curve, would be an estimate of the true mean, although less efficient[28] (p. 70). For reasons discussed earlier the recorded curve, however, is not a frequency function of transit times. If the record is interpreted according to ZIERLER[24], the meaning of the time defined by AUKLAND and WOLGAST is less clear.

Tracer dilution in renal vein. DEETJEN, BRECHTELSBAUER and KRAMER[21] studied dye dilution curves in the renal vein and, by exponential extrapolation of

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the first slope, partitioned the record into cortical and medullary components. Flow rates for cortex and medulla agreed with those calculated from measurements inside the tissue. Similar studies were reported also by REUBI, GOSSWEILER and GÜRTLER[29]. By using an ingenious technique OFSTAD, LUND-JOHANSEN and KOLSAKER[30] ascertained that in human kidneys systemic recirculation of dye does not interfere with the curve before it deviates from the first exponential downslope, but recirculation arrives before the extrapolated decline reaches the background level. GÓMEZ, DEMEESTER, STEINMETZ, LOWENSTEIN, SAMMONS, BALDWIN and CHASIS[31] applied to the renal circulation a mathematical technique for deriving a frequency distribution of specific blood flow from dye dilution curves[32]. Specific blood flow (i.e. flow per unit volume of blood) distribution curves did not seem to be much different from the normal curve; although both kurtosis and skewness were quantitatively determined, statistical significance of these deviations from the normal was not reported. A unimodal distribution of specific blood flow in the kidney may be a result of a wide dispersion of perfusion rates in the tissue, i.e. that differences in specific perfusion *within* the cortex and *within* the medulla are in magnitude similar to the difference *between* the average perfusion rates of the cortex and of the medulla.

External and internal monitoring of diffusible indicators. The inert gas washout technique of KETY[33] and the heated thermocouple principle[34] have also been applied to the determination of regional blood flow in the kidney. Transit of either radioactive Kr^{85} or Xe^{133} has been recorded externally[35–40], and with a detector inserted into the kidney[26]. Desaturation of H_2 gas[41] and dissipation of heat[42–44] have been monitored within the renal tissue. A very attractive feature of external monitoring is that the determination can be repeated, and changes in transplanted kidneys in dogs[37] and humans[38] have been followed by this technique over a period of several months. Uncertainty exists, however, regarding the assumptions and the procedure for extracting information from the records. In particular, difficulties arise in separating 4 exponential components[45] as the risks are considerable even when only 2 components are to be separated from data subject to experimental error[46,47]. A further problem with the technique is the presence of countercurrent exchange in the outer medulla since passage of diffusible substances through this region is hindered in both directions. Results can only be interpreted in a complex manner in terms of flow and efficiency of the countercurrent exchanger. Despite these difficulties, the results obtained with this technique for the normal kidney are in agreement with those of KRAMER and associates. In hemorrhagic hypotension a major and patchy reduction in cortical flow has been observed[36] while medullary flow was maintained at a relatively stable level. In a recent communication, BIRTCH, ZAKHEIM, JONES and BARGER studied the effect of furosemide and ethacrinic acid on renal flow distribution[48]. A decrease in outer medullary flow accompanied the diuretic effect; later, cortical flow increased above control values while the diuresis persisted.

Insertion of a detector into the renal parenchyma focuses the determination to a small and possibly homogeneous volume of tissue. AUKLAND, BOWER and BERLINER[49] and AUKLAND and BERLINER[41] studied hydrogen desaturation of various regions in the kidney by polarographic electrodes. They found that below the rate of 1.5 $ml \cdot min^{-1} \cdot g^{-1}$ directly measured renal venous outflow agreed with the cortical flow calculated from the H_2 curve. In the inner medulla a strong dependence of H_2 desaturation on urine flow was demonstrated. In the outer medulla, urine flow rate affected desaturation to a much lesser extent but, after full saturation of the kidney with H_2, washout did not follow a single exponential curve. In a recent communication AUKLAND and WOLGAST[26] reported to have overcome most of these difficulties by developing a technique for a reproducible partial saturation of the kidney with H_2. Under these conditions the outer

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medullary blood flow rates calculated from 3 separate measurements were in good agreement. These techniques were H_2 desaturation and transit measurements of Kr^{85} and of P^{32} tagged red cells with a semiconductor detector. In contrast to the findings of CARRIER et al.[36] and TRUNIGER et al.[40], AUKLAND and WOLGAST found a reduction in outer medullary blood flow during hypotensive periods.

AUKLAND[50] has studied the renal medullary heat clearance by infusing cold (20–25 °C) saline into the renal artery. He concluded that heat conduction from the medulla is probably the only factor responsible for the observed results and convective dissipation of heat by flows in vasa recta and loops of Henle need not be implicated.

SCHER[42] studied the effect of various drugs on focal circulation in the kidney with heated thermocouples. This technique was employed also by GRÄNGSJÖ, SANDBLOOM, ULFENDAHL and WOLGAST[43] and GRÄNGSJÖ[44] for measuring regional flow in the medulla. In these experiments a miniature assembly of a heating element and of 2 temperature measuring devices was placed into the tissue. According to the observations of the Swedish investigators convective flow dissipation under various experimental conditions followed the pattern of vasa recta flow observed by KRAMER et al.[14,20]. Water diuresis was an exception; in this case no increase in heat convection was recorded.

Extraction of PAH and Rb^{86}. REUBI[51] suggested that at low plasma levels PAH is fully extracted from cortical blood, and that PAH extraction and total blood flow allow the calculation of cortical and medullary flow rates. Although this premise may not be entirely valid, experimental evidence favors the view that a good qualitative estimate of changes in medullary blood flow can be obtained by this technique[52]. Factors that render this method only semiquantitative were reported by SCHNERMANN and THURAU[53], who found rediffusion of PAH from the collecting ducts into the medullary interstitium, and by NISSEN[54] who took advantage of an anatomical feature of cat kidneys that permits collection of purely cortical blood from superficial veins. NISSEN found an average PAH extraction of 95% from cortical plasma when the arterial plasma PAH level was below 4 mg/100 ml.

In a series of articles HÁRSING and associates[55–59] reported the application of SAPIRSTEIN's technique[60] to determining flow distribution in kidney. These authors found excellent agreement between rates of total blood flow determined directly by collecting renal venous blood and measured by Rb^{86} extraction. Distribution of blood flow through the kidney corresponded closely to values obtained by other investigators with different techniques. In osmotic diuresis equal fractions of PAH and Rb were extracted by the cortex; when the ureter was occluded, directly measured renal blood flow increased, but extraction of both substances diminished pari passu. The authors favor the explanation that under the condition of stopped urine flow a significant fraction of renal blood passed through arterio-venous shunts.

Recently, TAKÁCS and BENCSÁTH[61] used the Rb^{86} method in a study of the effect of denervation on intrarenal flow distribution. They found a substantial increase of medullary flow in the denervated kidney. This result provides experimental evidence for an earlier suggestion by BLAKE[62,63] that redistribution of blood flow may be instrumental in the production of polyuria which accompanies renal denervation.

Semiconductor radioactivity detectors. The development of miniature radioactivity detectors[64,65] has provided a new and valuable technique for local blood flow measurements. Circulation in brain[66] liver, and muscle[67] and kidney[6,7] has been studied by this method. The detector, a lithium activated silicon p-i-n type semiconductor, is sensitive primarily to β-radiation, but γ-rays are also detected although less efficiently. Radionuclides used so far include P^{32} [6] I^{131} [7] Au^{198} [67] Kr^{85} [26] and undoubtedly this list will be extended in the future. The smallest device is enclosed in a stainless steel tube, with a wall thickness of 50 μ, shaped as an 18 gauge hypodermic needle; larger detectors have also been used[27]. The output of the detector is usually connected to a ratemeter; recently, on-line computer analysis of the detector output has also been reported[68]. A particular advantage of this technique is that it allows the study of transit, accumulation and washout of tracers, many of which represent substances of physiological significance, in different parts of the kidney. Observations may be repeated within a limited time

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on the same kidney and, potentially, the results yield a measure of the dispersion as well as the average of flow rates. Insertion of the detector into the tissue causes some damage, an area of approximately 0.5–1.0 mm around the detector usually shows extravasated cells and torn blood vessels and tubules. In the experience of this writer presence of the needle in the tissue infrequently causes any change in urine excretion or in various clearance measurements. Tissue damage that occurs with the use of this type of detector has been discussed by WOLGAST[27] and by BROCK, INGVAR and SEM JACOBSEN[66].

WOLGAST[7] determined the transit times of P^{32} tagged erythrocytes in various regions of dog kidneys. He calculated 3 estimates for average transit times, i.e. area/peak, the mean and the median of the curves corrected for recirculation. He found only small differences between these estimates. In the cortex, mean transit time was between 2–3 sec. In the medulla, it increased from the corticomedullary border to the papilla from about 3–30 sec. Autoregulation was observed both in cortex and medulla and medullary mean transit time was shortened during mannitol diuresis. In addition to the experimental data, WOL-

Figs. 2–5. Oscilloscope screen photographs. Abscissa: time. Distance between columns of dots = 1 sec. Height of columns represent radioactivity (cps). As the light spot sweeps from left to right (of the reader) it is displaced vertically by a distance proportional to the count registered. The counter resets to zero after every 1 sec count and, without delay, starts anew. The upper edge of the white band connects average background counting rates (cps) obtained immediately before and after each run. Inset in the upper right hand corner of Figure 2 shows the longitudinal cross section of the experimental kidney. White loops enclose the cross section of the doughnut-shaped region around the detector within which about 2/3 of the P^{32} radioactive counts originated. Volume of I^{131} detection is larger and less circumscribed. (See text and [70].)

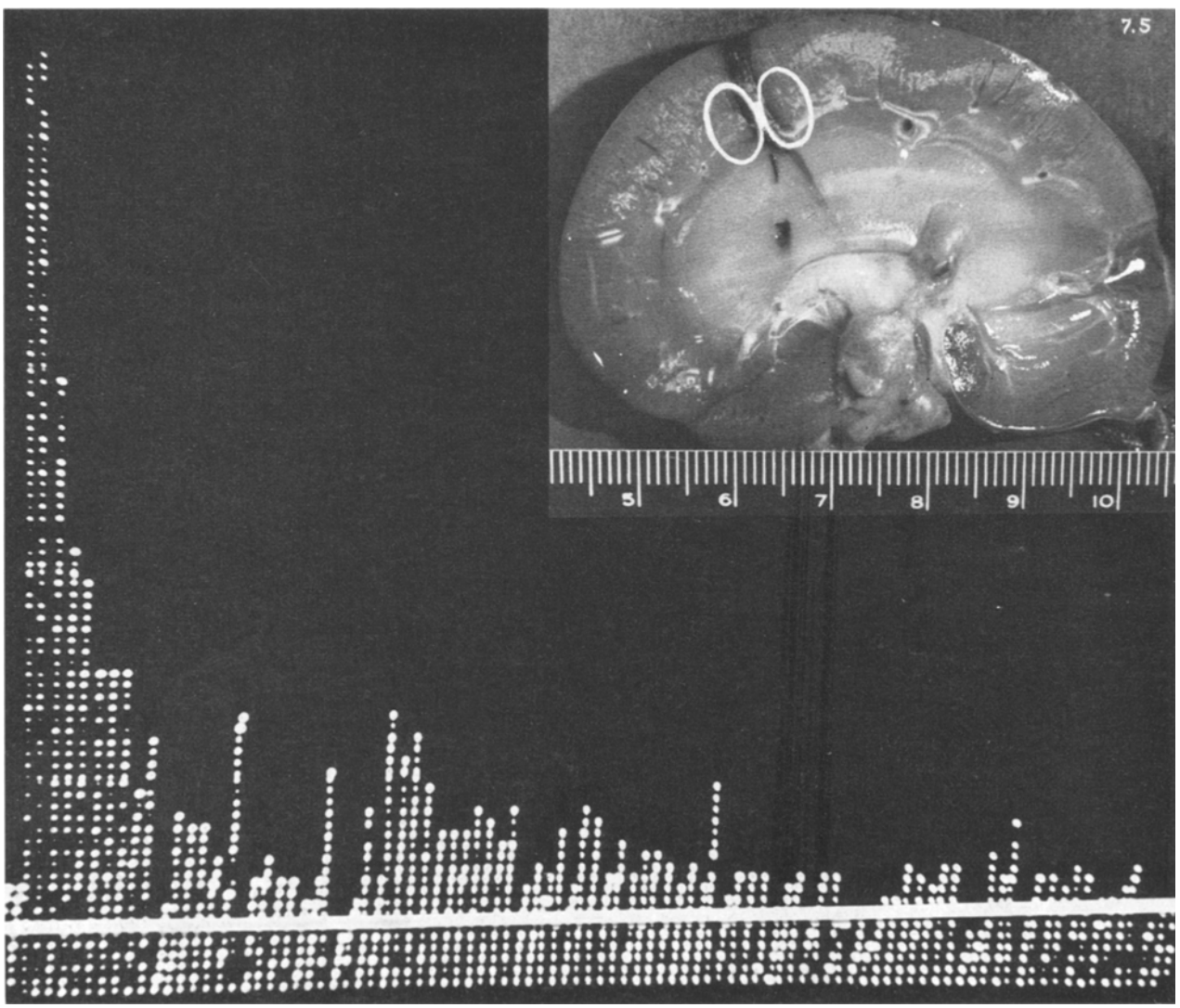

Fig. 2. Cortical transit of P^{32}-labeled red blood cells.

GAST's paper contains also a valuable discussion of the theoretical aspects of the method.

The present writer has also used a semiconductor device and measured the transit of P^{32} tagged red cells, I^{131} albumin and chromic phosphate – P^{32} (a colloidal suspension with average particle diameter of 0.05 μ) in dog kidneys[69]. Instrumentation and study of the geometrical efficiency of the detector have been published earlier[6,70]. Figures 2–5 represent selected[71] results from these experiments. The records were corrected for background activity and recirculation, and mean transit times were calculated according to ZIERLER[24] as A/P. In addition, the 17th, 50th and 83rd percentiles (P_{17}, P_{50}, P_{83}) were determined on the cumulative distribution plot and mean values were calculated by a formula of DIXON and MASSEY[28] (p. 404). As a measure of dispersion around the mean, P_{17} and P_{83} are also indicated. The data are shown in the Table.

Figure 2 shows transit of P^{32} tagged red blood cells through the cortex; the white loops in the inset enclose the region within which about $^2/_3$ of the total counts originated. Figure 3 indicates transit of labelled albumin through cortex. The parameters for cell and albumin transit through cortex are not directly comparable because they were obtained in different animals.

The figures for mean transit times through the cortex are longer than those of KRAMER and associates and of WOLGAST. It may be pertinent that extrapolation of the first exponential downslope was not used as correction for recirculation. A second wave of activity was eliminated by free hand extrapolation of the initial curvature.

[69] Some of these experiments were carried out in cooperation with Dr. E. M. GLASER. The author is grateful to Dr. D. B. ZILVERSMIT for suggesting the technique for labelling erythrocytes and to Dr. M. WOLGAST for recommending the use of chromic phosphate.

[70] G. G. PINTER, E. M. GLASER and S. KAHAN, Int. J. appl. Radiat. Isotopes *19*, 170 (1968).

[71] The condition of instantaneous input of tracer appears to be met if the peak develops rapidly after injection. In these cases the use of ZIERLER's formula for calculating $\bar{t}$ is applicable. Instantaneous input of tracer was seen in most cortical records, while it was observed infrequently in medullary experiments. Calculation of mean transit time from records with slowly developing rounded peaks must await further theoretical work.

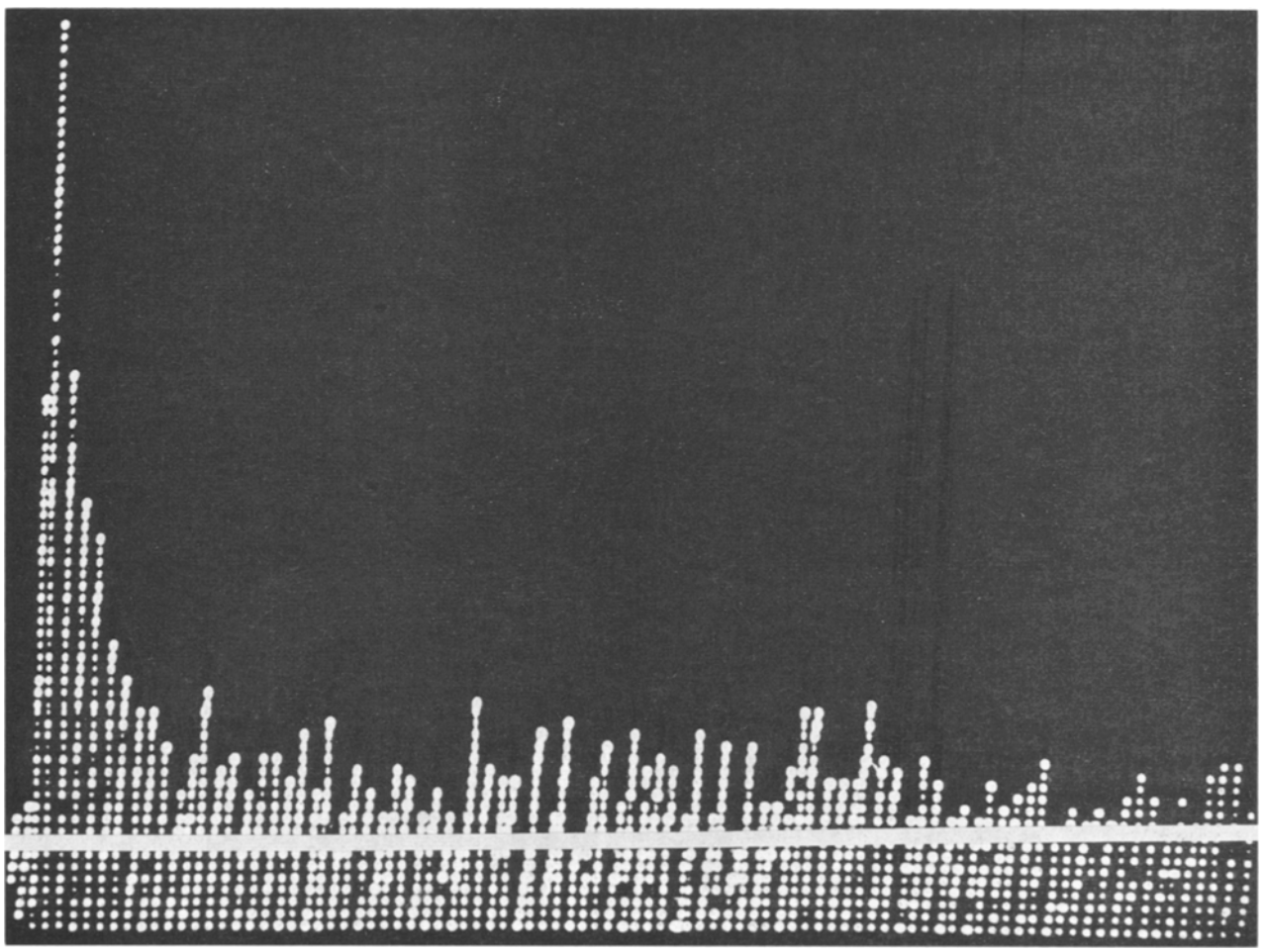

Fig. 3. Cortical transit of I^{131}-labeled albumin.

Figure 4 shows a record of inner medullary I^{131}-albumin transit in the same kidney in which Figure 3 was obtained. The animal received approximately 20 ml/kg of 0.5% saline in slow infusion prior to the tests and was producing hypotonic urine (U_{osm}/P_{osm} = 0.62). With the detector remaining in the same position acetylcholine was infused into the renal artery at a rate of 100 µg/min for 5 min, and a transit curve for I^{131}-albumin was again recorded (Figure 5). Acetylcholine infusion induced an approximately 3-fold reduction of the medullary mean transit time of plasma albumin. This coincided with an approximately 20% decrease in the equilibrium distribution of I^{131}-albumin. Therefore, an about 2.5-fold increase in medullary plasma flow occurred. A definite narrowing of the distribution of medullary transit times is also apparent.

The wide dispersion of transit times in both cortical and medullary tissue is conspicuous, and may have multiple causes. Firstly, random error originates from counting radioactivity for short periods. This factor, however, could not be a major one because administration of up to 5 times larger dose of P^{32} in the same volume of cell suspension in other experiments did not change the shape of the transit curve. Secondly, in the injured tissue around the detector capillary blood flow was abnormally slow. Again, this factor could not have contributed much to the wide dispersion of flow rates because the cortical record was the same regardless of whether the detector was placed over the cortical tissue or, later, inserted therein. Also, if slow flow in capillaries in the medulla was an artifact of tissue damage, it is not expected that infusion of acetylcholine would eliminate it. It seems, therefore, that a wide range of transit times in the renal cortex and medulla reflects an inherent wide dispersion of flow rates through capillaries and small blood vessels in these tissues.

In further studies the influence of osmotic diuresis on outer medullary transit of I^{131}-albumin was observed. An approximate decrease of 20% of $\bar{t}$ and a slight widening of the distribution of transit times accompanied a massive mannitol diuresis in this experiment. A study of the transit of cells and a colloidal suspension of chromic phosphate, both labeled with P^{32} and injected separately, indicated that perfusion of the inner medulla with red blood cells is relatively poor[72].

[72] G. G. Pinter, E. M. Glaser and C. C. C. O'Morchoe, 2nd Congr. Am. Soc. Nephrology, Abstracts p. 50 (1968).

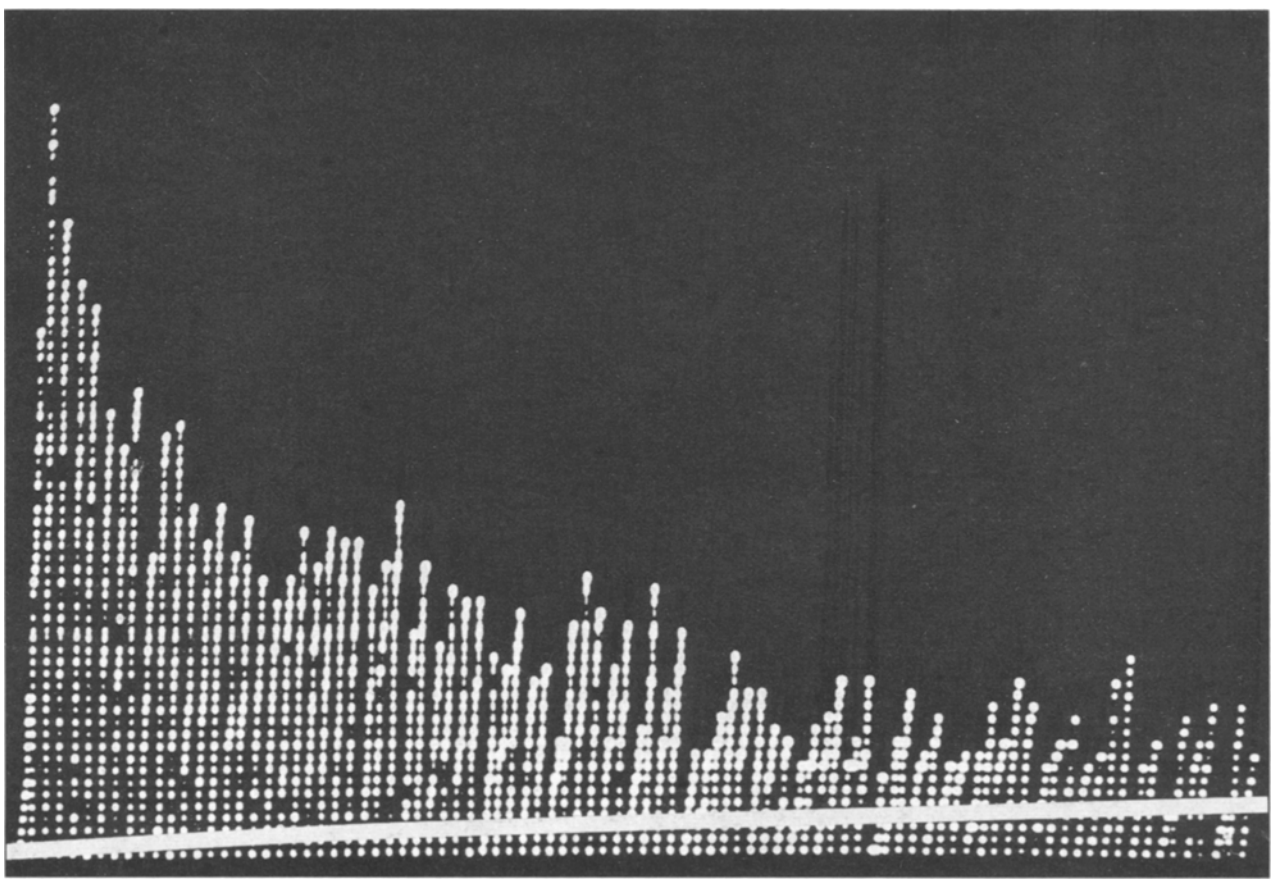

Fig. 4. Inner medullary transit of I^{131} albumin.

Conclusions

Although intensive interest in medullary blood flow is of relatively recent origin, many techniques have already been used to measure this important determinant of the urine concentrating process. Each method has its particular advantages and limitations. Usually a technique capable of providing more information is also apt to interfere more with the physiological state of the measured variable. A case in point is the needle detector which can gather information from a well circumscribed region but undoubtedly causes some tissue damage. In contrast, external monitoring techniques leave the tissue intact but may lead to difficulties in interpreting the data. By using both an adequate tracer and detector straightforward information can be obtained about blood flow through superficial tissue regions. In non-homogeneous tissues and, particularly, in regions lying below the surface the results may be obscured by a variable geometrical efficiency of the detector and a possible overlap in the distributions of transit times. Differences in mean perfusion rates can be discerned only if respective dispersions around the means are relatively narrow.

Owing to the complexity of the medullary circulation various techniques using tracers do not necessarily measure the same flow and even the same record may be interpreted in different ways. It is remarkable that in spite of the variety of measuring and calculating procedures, the values fall within a relatively narrow range.

	Area/peak	Mean (sec) $\frac{P_{17}+P_{50}+P_{83}}{3}$	$P_{17}-P_{18}$
Cortical RBC transit (Figure 2)	5.1	4.8	1.4– 9.4
Cortical albumin transit (Figure 3)	4.8	4.1	1.4– 8.0
Inner medullary albumin transit (Figure 4)	22.5	20.8	2.3–50.0
Inner medullary albumin transit after acetylcholine (Figure 5)	6.8	7.3	1.8–15.0

Means and dispersions of transit times. See text.

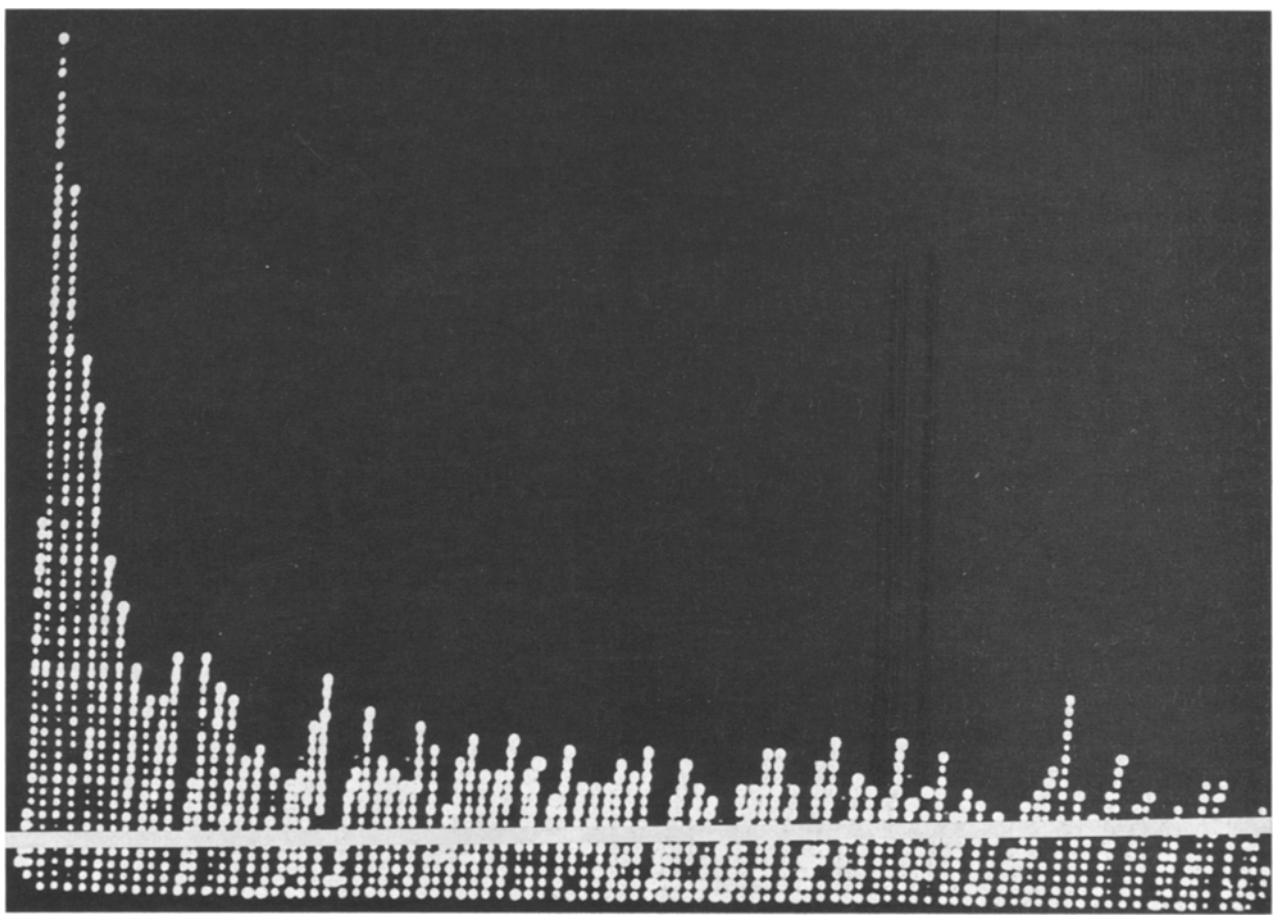

Fig. 5. Inner medullary transit of I^{131} albumin after infusion of 100 γ/g/min acetylcholine into the renal artery.

Clarification of many unresolved questions may be expected through an operational approach both in terms of laboratory and 'paper and pencil' operations[73]. Generally applicable theoretical formulae for calculating mean transit times and flow rates, and dispersions around the means are of the highest priority in future research on medullary blood flow. Miniature radioactivity detectors placed inside the tissue would seem to offer the best possibilities for further explorations. In these measurements P^{32} isotope has definite advantages: the observed tissue may be large enough to render insignificant the effect of the damaged segment around the detector and yet small enough to restrict the observation to a relatively homogeneous tissue. Further advances both in theory and applications of the tracer method should assist in integrating the knowledge pertaining to medullary blood flow and renal concentrating mechanism[74].

Résumé. Toutes les méthodes de détermination du flux sanguin dans la médullaire rénale sont basées, jusqu'à présent, sur l'emploi de substances traceuses. Les différentes techniques sont passées en revue dans le présent article. Quelques-unes manquent de fondation théorique rigoureuse; cependant, les valeurs moyennes obtenues pour le temps de passage à travers les zones corticales et médullaires ne varient que très peu.

Récemment, un petit détecteur de radioactivité enfermé dans une aiguille hypodermique a été introduit dans le parenchyme rénal et le passage d'isotopes radioactifs a été enregistré. Les résultats obtenus avec cette technique indiquent une large dispersion statistique du temps de passage de globules rouges marqués au P^{32} et d'albumine marquée à l'I^{131} dans les régions corticales et médullaires.

[73] P. W. BRIDGMAN, *The Nature of Thermodynamics* (Harper Bros., New York 1961).

[74] This work is supported by the National Science Foundation.

SPECIALIA

Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. – El responsable de los informes reducidos, está el autor.

The Synthesis of (±)-4-Methoxypterocarpin

(−)-4-Methoxypterocarpin (3,4-dimethoxy-8,9-methylenedioxypterocarpan) (I) was isolated from the heartwood of *Swartzia madagascariensis* Desv. (subfamily: Caesalpinioideae) by HARPER et al.[1] In previous papers[2,3], a new method for syntheses of pterocarpans (e.g. (±)-pterocarpin (II) and (±)-maackiain (III)) from a corresponding benzofurano[3′,2′:3,4]coumarin was reported. The present paper reports the total synthesis of (±)-I from 7,8-dimethoxy-4-hydroxycoumarin (IV)[4] according to the modified procedure described earlier[2,3].

By the procedure of WANZLICK's benzofurano[3′,2′:3,4]coumarin synthesis[5], the condensation of the 4-hydroxycoumarin IV with catechol gave 5′,6′-dihydroxy-7,8-dimethoxybenzofurano[3′,2′:3,4]coumarin (V, m.p. $>300°$), which was readily transformed to a diacetate (VI, m.p. 240–241°, IR 1760, 1744 cm^{-1} (acetate, α-pyrone) (Nujol), UV λ^{max}_{EtOH} nm (log ε): 242 (4.35), 266 (4.00), 332 (4.49), 348 (4.43). Found: C, 61.10; H, 3.96. $C_{21}H_{16}O_9$ requires: C, 61.17; H, 3.91%).

In a manner similar to the experiment described earlier[3,6,7], methylene iodide treatment of the 5′,6′-dihydroxy compound V gave a 5′,6′-methylenedioxycoumarin (VII, m.p. $>300°$, IR 1735 (α-pyrone), 1038, 934 cm^{-1} (O–CH_2–O) (Nujol), UV $\lambda^{max}_{CHCl_3}$ nm (log ε): 268 (3.95), 284 (3.93), 299 (3.91), 312 (3.98), 348 (4.47), 362_{sh} (4.42). Found: C, 63.61; H, 3.78. $C_{18}H_{12}O_7$ requires: C, 63.53; H, 3.55%). The reduction of the compound VII with lithium aluminum hydride yielded 2-(3,4-dimethoxy-2-hydroxyphenyl)-3-hydroxymethyl-5,6-methylenedioxybenzo[b]furan (VIII, m.p. 220–222°, UV λ^{max}_{EtOH} nm (log ε): 271 (4.16), 320 (4.38). Found: C, 62.90; H, 4.75. $C_{18}H_{16}O_7$ requires: C, 62.79; H, 4.68%). Its IR-spectrum exhibits absorptions due to hydroxyl groups at 3450 and 3200 cm^{-1}. Acetic anhydride-pyridine treatment of the compound VIII furnished a diacetate (m.p. 189.5–191°. Found: C, 61.66; H, 4.70. $C_{22}H_{20}O_9$ requires: C, 61.68; H, 4.71%). The compound VIII was dehydrated in boiling diethylene glycol to give an ether, 3,4-dimethoxy-8,9-methylenedioxy-6a,11a-dehydropterocarpan (IX, m.p. 187–189°, IR 1655, 1610, 1500 cm^{-1} (C=C, phenyl)

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