Table 2. EFFECT OF VARIOUS INHIBITORS ON ACTIVE D-XYLOSE TRANSPORT BY RAT MUCOSAL TISSUE IN A SODIUM AND A SODIUM SUBSTITUTED LITHIUM MEDIUM

Inhibitor	Conc. of inhibitor (mmolar)	Number of animals	Final D-xylose conc. in mucosal tissue (mmolar)	*T/M
Na^+ bicarbonate saline medium				
None	—	12	12·64 ± 0·57	1·90 ± 0·09
Phlorizin	0·1	4	6·96 ± 0·17	1·04 ± 0·03
Iodoacetic acid	12·0	5	7·45 ± 0·16	1·12 ± 0·03
Sodium fluoride	12·0	6	7·13 ± 0·32	1·07 ± 0·05
2,4-Dinitrophenol	0·5	4	7·13 ± 0·32	1·07 ± 0·05
Oligomycin	0·026	8	7·43 ± 0·30	1·12 ± 0·05
Li^+ bicarbonate saline medium				
None	—	8	10·20 ± 0·71	1·53 ± 0·11
Phlorizin	0·1	4	6·23 ± 0·37	0·94 ± 0·06
Iodoacetic acid	12·0	4	6·80 ± 0·28	1·02 ± 0·04
Lithium fluoride	12·0	4	6·80 ± 0·12	1·02 ± 0·02
2,4-Dinitrophenol	0·5	4	6·43 ± 0·25	0·97 ± 0·04
Oligomycin	0·026	11	8·67 ± 0·14	1·30 ± 0·02

The experimental conditions were the same as those for Table 1. D-xylose and inhibitors were placed on both sides of the tissue. The initial D-xylose concentration was 6·66 mmolar. The *S.E.* of the mean is indicated for each result.

* Ratio of final D-xylose concentration in mucosal tissue water to final D-xylose concentration in the mucosal incubation medium.

under this condition completely inhibits active D-xylose transport.

These experiments illustrate that the mechanism of active D-xylose transport by mucosa from rat jejunum is similar in either sodium or lithium bicarbonate saline. In addition, the energy requirements for this process are similar to those observed for the active intestinal transport of other sugars[15,16]. The fact that lithium can replace sodium in the incubation medium is the only apparent difference in the mechanism of active transport of this pentose and other sugars.

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Identification of Prostaglandin E_2 as the Principal Vasodepressor Lipid of Rabbit Renal Medulla

INVESTIGATORS in several laboratories have reported on the isolation of vasoactive lipids from renal medulla of several species[1–7]. Muirhead *et al.*[1] have separated these lipids into two classes: the antihypertensive lipids which prevent canine renoprival hypertension[2] and lower canine renal hypertension[3]; and the more acidic vasodepressor lipids which acutely lower the blood pressure of the anaesthetized, vagotomized dog or rat treated with pentolinium[4].

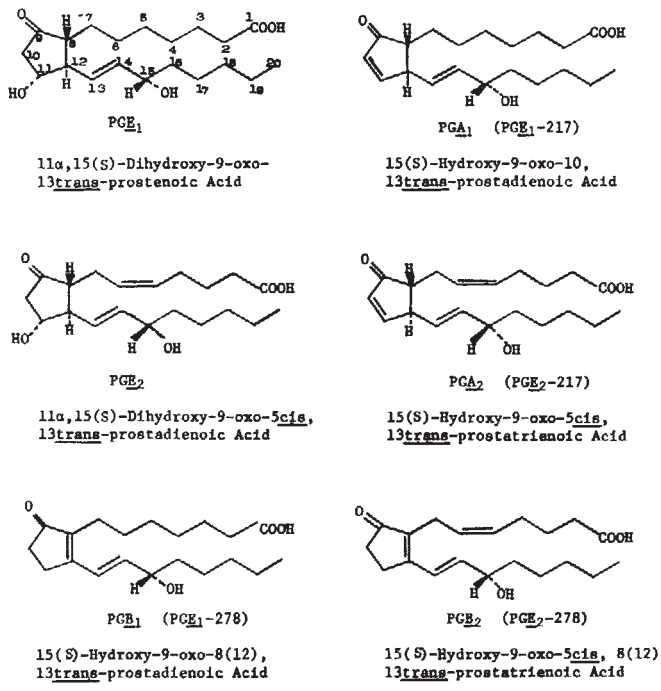


Fig. 1.

Hickler *et al.*[5] suggested that the acidic depressor lipids in extracts of renal medulla are prostaglandins[6]. In a similar study Lee *et al.*[6] separated two depressor lipids, one tentatively identified as PGE_1 (Fig. 1). The other depressor lipid differed chromatographically and was unable to stimulate non-vascular smooth muscle and was named "medullin". Subsequently, this material was identified[9] as PGE_2-217 (now termed PGA_2 (ref. 10)). Strong *et al.*[7] also isolated vasodepressor lipids from rabbit renal medulla and interpreted their findings to indicate that their "purified VDL" was PGE_1. They pointed out the possibility of the formation of "medullin" from PGE_1 by acid treatment and the similarity in the properties of "medullin" and PGE_1-217 (PGA_1) (ref. 11).

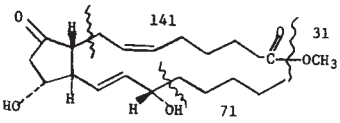
This communication concerns the identification of the acidic depressor lipids from rabbit renal medulla using less equivocal methods. Five kilograms of rabbit renal medulla were homogenized for 5 min in a Waring blender with an equal volume of water (*p*H adjusted to 7·0). After the addition of three volumes of acetone, the mixture was stirred and then allowed to settle overnight at 4° C. The insoluble residue was removed by filtration and the filtrate (75 per cent acetone) was concentrated *in vacuo* to an aqueous extract containing 10–20 per cent acetone. The solution, still at *p*H 7, was extracted twice with equal volumes of petroleum ether (boiling point 30°–60° C). The aqueous phase was adjusted to *p*H 3·0 with hydrochloric acid and extracted three times with 1/4 volume of methylene chloride. These three phases were combined and concentrated to approximately 1 l. The combined methylene chloride solution was extracted four times with 1/4 volume of 0·2 molar phosphate buffer, *p*H 8·0. After evaporation to dryness the methylene chloride layer provided the "neutral" fraction which was reserved for other studies. The aqueous buffer phase was acidified to *p*H 3·0 with hydrochloric acid and extracted three times with 1/3 volume of methylene chloride. After evaporation of the solvent, 0·35 g of acidic lipid fraction was obtained. All the activity detectable by the acute vasodepressor assay[4] was found in this acidic fraction.

Silicic acid chromatography[7] of pooled acidic lipids (984 mg) provided only two depressor fractions which were predominantly *E* (103 mg) and *A* (13 mg) prostaglandins. Thin-layer chromatography of the PG*A* fraction and subsequent elution with methanol from the silica gel[12] impregnated with silver nitrate plates indicated that PGA_2 was the principal component of this fraction. The behaviour of this sample on thin-layer chromatography compared with an authentic sample and the conversion by alkali degradation to PGB_2 (Fig. 1) were the signs used to identify this material.

Preparative thin-layer chromatography of 50 mg of the PG*E* fraction from the silicic acid column on a silica gel plate (20 × 20 cm) impregnated with silver nitrate 1 mm thick, developed with chloroform : methanol : acetic acid (CMA) (90 : 5 : 5), yielded 31 mg from the PGE_2 zone on elution with methanol. PGE_1, if present at all, constituted less than 10 per cent of the PG*E* content of the renal medulla.

A portion of the PGE_2 eluted from the chromatographic plate was esterified with ethereal diazomethane. The resulting methyl ester was shown by thin-layer chromatography and mass spectrometry to be identical to an authentic sample of PGE_2 methyl ester (Systems *M*I, *M*II, *M*III (ref. 13) (Table 1)). In still further confirmation of its identity another portion of the PGE_2 was treated with alkali and converted to PGB_2 which exhibits strong ultra-violet absorption at 278 mμ and has thin-layer chromatographic mobility distinguishable from that of PGB_1.

Table 1. MASS SPECTRAL DATA FOR PGE_2 METHYL ESTER



	M⁺	*M*−18	*M*−(2×18)	*M*−(18+31)	*M*−71	*M*−(18+71)
m/e	366	348	330	317	295	277
Per cent of base peak	1·0	21·0	11·6	6·7	10·9	21·0

	M−(18+140*)	*M*−(36+140*)
m/e	208	190
Per cent of base peak	56·7	44·7

* Fragmentation involves hydrogen transfer.

In view of these findings it would appear that there is little doubt that the principal vasodepressor lipid of rabbit renal medulla is PGE_2. This is consistent with reports that the PGE_2 precursor, arachidonic acid, is present in much larger quantities than the PGE_1 precursor, dihomo-γ-linolenic acid, in renal medulla[14].

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IMMUNOLOGY

Interferon and the Interaction of Allogeneic Normal and Immune Lymphocytes with *L*-cells

IT has been supposed[1–3] that the effect of immune lymphocytes is connected with the transmission between the lymphocytes and target cells of the informative active parts. The destruction of homologous cells induced by lymphocytes is similar to the primary cytopathic effect in virus diseases. Nuclei of immune lymphocytes proved to exert the same destructive effect on target cells as that of intact immune lymphocytes[2,3].

Our histochemical and autoradiographic investigations have demonstrated that cytoplasmic connexions ("bridges") containing DNA and RNA are formed between lymphocytes and target cells, and that H^3-uridine-labelled RNA of lymphocytes and numerous granules containing DNA appear in the cytoplasm of *L*-cells. These processes are more marked with immune lymphocytes than with normal allogeneic lymphocytes of *BALB/c* mice[4].

We consider it possible that interferon is produced in the course of immune lymphocyte target cell interaction; and that the interferon induced by virus should suppress that action of immune lymphocytes.

In experiments carried out with Dr. Inessa A. Svet-Moldavskaya, we attempted to detect interferon production in the course of interaction of immune lymphocytes *BALB/c* with *L*-cells. Interferon was tested by means of variola virus plaque formation in *L*-cells culture. All these experiments produced negative results. Investigation of virus interferon, however, revealed an unexpected result.

Continuous *L*-fibroblasts were used as target cells to which immune or normal lymphocytes from *BALB/c* mice were added (Rosenau and Moon's system[5]). *BALB/c* mice were immunized twice intraperitoneally with $2{\cdot}5 \times 10^6$ *L*-cells at an interval of one month, and one month later the mice were inoculated into the spleen with $2{\cdot}5 \times 10^6$ *L*-cells in 0·1 ml. Four days after inoculation the spleens were removed aseptically and made into a suspension in medium 199 by means of Potter's homogenizer. In the same way suspensions of cells were prepared from the spleens of non-immunized *BALB/c*. The cells were filtered and the numbers of live lymphoid cells were counted.

Interferon was obtained according to Hare and Morgan[6]. The chick embryo allantoic fluid containing Newcastle disease virus was irradiated with ultra-violet light until the complete loss of infectivity to chick embryos. The allantoic fluid of normal chick embryos was irradiated in the same way. Each of these fluids was diluted 1 : 20 with medium 199 and was then added to monolayer *L*-cell cultures. Cultured fluids from *L*-cells treated both