RESPONSE OF THE CONJUNCTIVAL VESSELS OF DIABETICS TO "PRISCOL"

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The accessibility of the human conjunctiva to direct examination has led many workers to study the structure and behaviour of its vessels in life (Graves, 1934; Lack *et al.*, 1949; Ditzel and Sagild, 1954). Not unreasonably, it has been suggested that certain general vascular abnormalities might reveal themselves in disordered structure or function at this site. The present study was based upon such a suggestion made by N. Ashton and C. A. G. Cook (1954, personal communication); they suspected, from clinical observations, that the conjunctival vasodilatation caused by "priscol" (tolazoline hydrochloride) eye drops was much less conspicuous in diabetics with retinopathy than in diabetics with normal fundi. These observations suggested to them the possibility of a circulating vasoconstrictor substance influencing the conjunctival vessels of the patients with retinopathy and, perhaps, also responsible for constriction of the pre-capillary arterioles of the retina with stasis and micro-aneurysm formation distally (Ashton, 1953).

We tested the reality of the clinical impression when the opportunity arose to examine a large number of eyes in an extensive study of diabetic retinopathy.

Method

Priscol, probably acting directly upon the vessel wall (Goodman and Gilman, 1955), causes striking reddening of the normal eye when strong solutions are put into the conjunctival sac (Fig. 1). Two concentrations of the

FIG. 1.—The conjunctival vessels before and 5 minutes after instilling priscol.

drug, 1/500 and 1/650 in sterile distilled water, were selected by a trial-and-error method as being adequate to produce conjunctival vasodilatation in most but not all normal eyes and thus likely to display a variation in response in the patients. Distilled water alone produced no appreciable vascular effect at five minutes.

Both eyes of 297 patients of the Diabetic Clinic at St. Mary's Hospital were studied. Table I shows the age and sex composition of the group. Each patient's

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eyes were first examined with the slit-lamp and corneal microscope, and those with corneal or conjunctival disease were excluded. These exclusions, inadequate record, and occasional patients with an eye removed, account for the small disparities between the number of patients in the group and the total number of eyes examined. One drop of the 1/500 priscol solution was then placed in one eye, usually the right, and one drop of the 1/650 solution in the other. The eyes were again inspected after a lapse of five minutes and the degree of reddening in each was recorded on an arbitrary scale of from nil to three plus (0, +, ++, +++). Throughout the study, the instilling of drops and the reading of the response were done by the same observer (R.S.), who, later, also inspected the fundi.

TABLE I.—Age and Sex of Diabetics Examined

	20–	30–	40–	50–	60–	70–	Total
Male	5	16	22	25	27	12	107
Female	16	10	17	47	64	36	190
Total	21	26	39	72	91	48	297

TABLE II.—Comparison of Responses at the Two Concentrations

	0	+	++	+++	Total	Mean Response
1/500 { No. ..	18	89	106	82	295	1.854
% ..	6.1	30.2	35.9	27.8	100	
1/650 { No. ..	33	101	98	46	278	1.565
% ..	11.9	36.4	35.2	16.5	100	

$$\chi^2 = 15.049. \quad n = 3. \quad P < 0.01 > 0.001.$$

Comparison of the Two Concentrations

Table II shows the incidence of the various grades of response at the two concentrations. If these arbitrary grades are treated numerically by making each plus value count one, the mean response at the higher concentration is 1.854, a value significantly greater than that at the lower concentration, 1.565 ($p < 0.01$). Comparison of the distribution of responses at the two concentrations also demonstrates a highly significant difference between them ($\chi^2 = 15.049$, $p < 0.01$).

Influence of Age and Sex upon Response

A clear relationship between the age and sex of the subject and the response to priscol can be seen in Fig. 2, in which the mean priscol responses for each 10-year age group, calculated separately for men and women (Table III), have been plotted. The response increases with age, and is greater in males than in females at all ages. The straight lines represent the calculated regression of response upon age; the degree of correlation between the two factors, though small, is significant in both sexes at the 1/500 concentration (men: $r = 0.240$, $p < 0.05$; women: $r = 0.368$, $p < 0.05$). At the 1/650 concentration, as expected, the mean values of response are, age for age, lower

TABLE III.—Priscol Response (Means) by Age and Sex

	20–	30–	40–	50–	60–	70–	r	p
1/500	M. ..	1.33	1.75	1.68	2.16	2.18	0.240	<0.05
	F. ..	0.85	1.30	1.47	1.89	1.92	0.368	<0.05
	All	0.94	1.58	1.59	1.99	2.00	2.13	
1/650	M. ..	0.67	1.80	1.40	2.05	1.92	0.202	<0.1
	F. ..	0.69	1.13	0.88	1.59	1.58	0.237	<0.05
	All	0.67	1.56	1.16	1.74	1.68	1.77	

than at 1/500 (Table III); the sex difference and the increase with age are shown in Fig. 3. The correlation between response and age, though significant in the women, just fails to achieve significance at the 1 in 20 level in the men (men: $r=0.202$, $p < 0.1$; women: $r=0.237$, $p < 0.05$).

Further Analysis of the Age-response Relationship

The correlation with age is a statistical phenomenon only, casting no direct light upon the precise biological mechanism determining the change. We sought further leads by examining the priscol response in relation to two characteristics, themselves related to age—namely, the duration of the diabetes and the arterial blood pressure.

FIG. 2.—Response to 1/500 concentration of priscol in relation to age and sex.

FIG. 3.—Response to 1/650 concentration of priscol.

TABLE IV.—Priscol Response (Means) by Duration of Diabetes

	0-	5-	10-	15-	20-	25+	Total
1/500 { No. ...	176	53	27	24	10	5	
Mean response	1.90	1.81	1.67	2.00	1.70	1.20	295
1/650 { No. ...	169	47	24	24	10	4	
Mean response	1.61	1.42	1.54	1.79	1.30	0.75	278

Influence of Duration of Diabetes.—In Table IV, the mean priscol responses are shown for each five-year duration group. The relationship between these two factors is poor, and statistically is not significant (1/500 $r=0.039$; 1/650 $r=0.015$).

Influence of Blood-pressure.—Since Hamilton *et al.* (1954a) have shown that arterial blood-pressure rises with age, a correlation between the priscol response and the blood-pressure might be expected simply on the basis of a common effect of age upon both. Age is not the only factor that influences the blood-pressure, for a considerable variation in pressures about the mean is found at all ages (Hamilton *et al.*, 1954a). Though in order to seek a relationship between pressure and priscol response independently of the effects of age it might at first sight appear necessary to examine each age group separately, it is possible to make suitable statistical adjustment to pressures, so as to remove only that part of the variation due to the effect of sex and age; correlations with the residual variation can then be sought. We have used the method of adjustment and the tables of Hamilton *et al.* (1954b) and calculated age-and-sex-adjusted blood-pressure score for each patient; the score represents the deviation of an individual pressure from its sex- and age-group mean, adjusted with regard to the increasing variation of blood-pressure with age to a corresponding deviation at 60 years of age.

When these scores, systolic and diastolic, were studied in relation to the priscol responses, no significant correlation between the two was found.

	1/500	1/650
Systolic ...	$r=0.059$	$r=-0.016$
Diastolic ...	$r=-0.045$	$r=0.026$

Influence of Retinopathy upon Priscol Response

In order to examine the original contention of a lessened response to priscol in diabetics with retinopathy, patients with cataract were excluded from

TABLE V.—Priscol Response and Retinopathy

Retinopathy Group	1/500 Priscol Response					1/650 Priscol Response				
	0	+	++	+++	Total	0	+	++	+++	Total
1	10	56	62	52	180	19	65	56	33	173
2	1	14	24	12	51	2	15	23	5	45
3	2	5	9	4	20	4	6	6	3	19
4	5	10	7	5	27	6	11	8	1	26
Total	18	85	102	73	278	31	97	93	42	263

$\chi^2=13.50$. $n=9$. $P=<0.2>0.1$. $\chi^2=15.38$. $n=9$. $P=<0.1>0.05$.

the analysis and the remainder divided into four groups on the basis of the appearance of the fundi. Group 1 patients had no micro-aneurysms (defined as discrete red dots); group 2 had up to 5; group 3 had 6 to 10; group 4 had more than 10. The distribution of the various grades of priscol response in these retinopathy groups is shown in Table V; the results of the χ^2

analyses indicate the absence of a relationship between the two factors. This is confirmed by the results shown in Table VI, where it can be seen that the proportion of poor priscol responses (0 and +) was the same whether retinopathy was present or not.

TABLE VI.—*Priscol Response and Retinopathy*

Retinopathy	1/500 Priscol Response		1/650 Priscol Response	
	Weak	Strong	Weak	Strong
Absent	36.6%	63.4%	48.5%	51.5%
Present	37.8%	62.2%	48.9%	51.1%

Discussion

The effects of priscol upon the blood vessels of diabetic patients have been studied by many workers; evidence for impairment of priscol-induced vasodilatation has been demonstrated in the feet by Handelsman *et al.* (1952) and in the retinal vessels by Cholst *et al.* (1952).

Several factors may influence the intensity of the effect of a priscol solution upon the conjunctival vessels, including the concentration of the substance, its penetration through the conjunctival epithelium, the responsiveness of the vessel wall, and the rate of destruction, dilution, and removal from the eye. Comparison of the effects of the 1/500 with the 1/650 concentration show that quite small differences in strength produce a perceptible difference in effect; this finding, however, would be considered better established if the eyes had been assessed quite independently of each other.

The sex difference and the age trend in response can, however, be accepted with confidence, especially since they were completely unforeseen and emerged only at the final analysis of results. It has been shown that duration of the diabetes and the level of arterial blood-pressure are unrelated to these trends. We also failed to find evidence that the response was in any way influenced by the presence of diabetic retinopathy, an observation in accord with that of Bárány (1955), who found no greater diminution of heat elimination from the skin blood vessels of diabetics with retinopathy than from those without it. Cholst *et al.* (1952), however, claimed that, when retinopathy was present, priscol did not produce normal retinal vasodilatation, which they assessed by the increase in width of the scotoma cast upon the visual fields by the retinal vessels.

A possible explanation of our findings may lie in de Roeth's (1953) investigations into the influence of age and sex upon the rate of tear production. Fig. 4, which is derived from his data, shows the mean rate of tear production in 10-year age-groups for normal men and women. Tear production was estimated by the method of Schirmer (1903), measuring the wetted length of a filter-paper strip of standard width five minutes after insertion into the lower conjunctival fornix. The figure shows a clear tendency for tear production to decrease with age, and, at most ages, to be less in men than in women. Assuming that sex and age exert a similar influence upon the rate of tear production in the diabetic, de Roeth's results suggest the simplest explanation for our findings; for the higher the tear flow, the more effective would be the dilution and washing out of priscol. This would account both for the lower priscol response in women, and the increase in response with age. The most intense response to priscol

vasodilatation which we saw was in a middle-aged woman with kerato-conjunctivitis sicca who was excluded from the analysis because of this condition.

If, in fact, the increase of effect with age is due to the decline in tear production, then the practical implications may be wide: a similar influence may affect other substances placed in the conjunctival sac for diagnostic, therapeutic, and experimental purposes. It may, for example, partly explain the widely accepted clinical observation that the mydriatic and cycloplegic drugs used locally in diagnostic procedures act less efficiently in the young than in the old. The employment of a local analgesic to potentiate the action of such drugs may well owe its effect not only to the synergic action and an increase in corneal permeability, but also, as demonstrated by Schirmer (1903), to inhibition of the lacrimatory reflex. The ophthalmoreaction of Calmette (1907), provoked by instilling tuberculin into the eye, enjoyed a brief vogue as a diagnostic test for

FIG. 4.—Mean rate of tear production in 10-year age groups of normal subjects. (From de Roeth's data.)

tuberculosis; its abandonment was due not only to the occasional unpleasant sequels in the eye but also to the lack of reliability of the response; one astute observer ascribed his negative results in children to the possibility that "tears washed out the testing fluid" (Woodcock, 1908).

Therapeutically, the findings would seem to indicate the need to consider age and sex in relation to the frequency of application and the concentration of eye-drops. Furthermore, while in the young the effects of drugs upon the eye may be lessened by the greater tear-flow, the systemic effects may be correspondingly enhanced by more rapid alimentary absorption.

Variation in tear production is clearly of importance in the interpretation of experimental studies upon the conjunctival response to drugs. Ridley (1932) timed the disappearance of coloured solutions of differing concentration from the eye, and hence calculated the rate of tear production. His general conclusions display no awareness of variation with age and sex. Where the effects of two substances are being compared, or when an agent is being compared in two groups of people, false conclusions may readily be drawn if the sex and age of the subjects are not considered. Lee and Holze (1950, 1951) examined the response of the conjunctival vessels to locally applied adrenaline, and claimed that

the vasoconstriction which it produced was greater in patients with essential hypertension than in normal controls. Their controls, however, were healthy young subjects whose relative lack of response may thus quite possibly have been due merely to greater dilution and washing out of the adrenaline by tears.

Conclusions

The vascular response of the conjunctiva to locally instilled priscol has been recorded in 297 diabetics.

Analysis shows a significant correlation between this response and the concentration of the drug, and also with sex and age.

There was no relationship between the response and the adjusted blood-pressure score, the duration of diabetes, or the presence of retinal micro-aneurysms.

This variation of the response with age and sex may be related to the rate of tear production, which, in normals, has been shown to be greater in females and to decrease with age.

The diagnostic, therapeutic, and experimental implications of these findings are considered.

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"It is this matter of 'communication' which lies at the root of the National Council of Social Service's work; and assumes a greater importance as the machinery of the Welfare State is elaborated and its philosophy is developed. For there seems to be opposition between these two things. Each year sees additions to the already complicated structure which the State is erecting for welfare, education, health, and so on; and with every new job, defined in terms of duties and responsibilities over against a pre-existing job, the problem of communication is increased by the erection of new invisible boundaries. Yet the philosophy which seems at the same time to guide all present-day social thinking is based on the need for coming together, for community responsibility, for bringing our social misfits out of their specialized institutions into the everyday world. The whole structure of the National Council was designed to make easier this communication between the national voluntary organizations among themselves and with the statutory bodies, both at the centre and, by means of local groups, throughout the country. Some of the ways in which the Council's departments and associated groups have, in their different but interdependent fields, worked towards that purpose during the past year are shown in this year's report." (40th Annual Report, National Council of Social Service.)

PROTEINS OF THE LIQUOR AMNII

BY

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Despite all the available information concerning the composition, origin, and fate of the liquor amnii, our knowledge with regard to this fluid remains incomplete. This communication presents an evaluation of the results obtained with paper electrophoresis of the protein pattern in the amniotic fluid, maternal serum, umbilical cord blood, and *in vitro* dialysates. The study was primarily undertaken for the purpose of obtaining further data on the possible source, nature, and function of the protein in the liquor amnii. It was also hoped that it might elucidate certain important and related clinical problems, such as the nutritional value of the swallowed liquor to the foetus and the possible diagnostic value of any detectable atypical electrophoretic pattern in abnormal pregnancies.

A review of the meagre literature on electrophoresis of the liquor (McCarthy and Kekwick, 1949; Bramwell *et al.*, 1953; Candiani, 1956; Wirtschafter and Williams, 1957a, 1957b; McKay *et al.*, 1958) shows that much of this work has been done on experimental animals. These studies have yielded some interesting information. For instance, in rats Wirtschafter and Williams (1957a, 1957b) have shown that in contrast to the maternal serum the albumin/globulin ratio in the liquor of these animals is subject to characteristic fluctuations. High albumin readings were noted at parturition in normal cases and a correspondingly high globulin value in abnormal ones. Brambell *et al.* (1953), on the other hand, reported that β - and γ -globulin in immune rabbit serum enter foetal circulation when such a serum is injected into the pregnant uterine lumen. The clinical significance of such findings, if observed in the human, could be of inestimable value, but the only two available studies on the liquor in man (Candiani, 1956; McKay *et al.*, 1958) have been controversial and not entirely in agreement with one another. In view of this, and because of the primary role of protein in foetal metabolism, it was felt that a further study in this field might prove instructive.

Material and Method

Samples of liquor amnii and venous blood were collected from 11 essentially normal patients who were surgically induced at 40 weeks plus five days. To avoid contamination all samples of amniotic fluid were withdrawn from the hind-water with a Drew Smythe catheter.

In seven additional cases enough maternal blood was removed to provide an adequate quantity of serum for *in vitro* dialysis against normal saline across their respective "foetal" membranes. The membranes utilized in the dialysis were in each case selected from an area about $\frac{1}{4}$ in. (1.3 cm.) beyond the placental edge. Care was also taken to ensure that each specimen included both amniotic and chorionic layers. The electrophoretic findings in the resultant saline dialysate