Congo Red Test for the Diagnosis of Protein Loss through the Gastrointestinal Mucosa

By

O. Saxl

Clinical Unit of the Paediatric Research Institute, Brno, Czechoslovakia

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The explanation of protein losing enteropathy by Citrin et al. [6] and other authors [1, 10, 13, 14, 17, 18, 21], proving that proteins are lost by way of the digestive system, is one of the important recent discoveries in the field of digestive pathology. Severe hypoproteinaemia of known origin is sometimes combined with losses into the digestive system which could be caused by intestinal mucous membrane oedema. This may give rise to a vicious circle, which, if not interrupted, leads irreversibly to death.

According to Jeffries et al. [13] the mechanism involved in the passage of plasma proteins across the gastrointestinal mucosa may be explained as follows.

(i) Passive diffusion between mucosal cells

(ii) Active secretion by mucosal cells

(iii) Exudation through inflamed or ulcerated mucosa

(iv) Loss secondary to disturbed mucosa cell metabolism

To demonstrate protein losses, albumin-bound radioactive iodine [6] or chromium [24] has been used in addition to radioactive polyvinylpyrrolidone [9] and dextran [19]. Recently, albumin-bound iodine combined with oral amberlite [12], to avoid reabsorption, has been put to trial. Neither the efficiency nor even the necessity of this method may satisfactorily be evaluated without repeating the test several times. It is thus impossible to establish whether the method is absolutely harmless, particularly in the case of young infants. In addition, it is difficult to perform these complicated tests in the hospital laboratory and this is the case also with immunological examinations. The only simple procedure is that of Jones [14], to determine intestinal protein losses from the amount of nitrogen excreted with urine.

The intestinal excretion of non-radioactive PVP was demonstrated by Bosseckert and Werner [4], by Schubert's method [22]. However, when using PVP or dextran, the possibility of these substances being phagocyted by, and causing impairment or even blockade of, the reti-

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culoendothelial system must be borne in mind. Besides, even PVP may be partly reabsorbed [1].

According to HITZIG [10], albumin tends to bind acid and alkaline dyes. This made us to search for a dye suitable for demonstrating protein losses. Evans blue, Coomassie blue, methylene blue and fluorescein failed to meet requirements, but Congo red seemed to fulfil the purpose. This dye was administered by BÜRKMANN and SCHLEICHER [5] to a patient suffering from amyloidosis with protein losing enteropathy, and he was found to evacuate red coloured stools repeatedly. This staining was not evident in any of the controls, so the authors suggested that the Congo red was bound by albumin and thus lost into the intestines. That Congo red is really bound to protein, up to a proportion of 1 : 1 to albumin and at higher concentrations also to globulin, has been demonstrated by BENNHOLD et al. [3]. These authors could show that albumin bound Congo red, but also other dyes and drugs, had identical diffusional and electrophoretic characteristics as the protein itself to which they were bound; this makes it possible to identify the fraction. We have used this method with success.

METHOD

A freshly prepared 1.5—3 per cent solution of Congo red in sterile water [11] was used. Of this 0.25 ml per kg body weight was applied intravenously. The maximum dose for adults was 18 ml. Before the test an enema was given and the subsequent three stools were examined.

RESULTS AND DISCUSSION

The Congo red test was performed 91 times in 82 children; some of them were suffering from diseases in which a pathological permeability of the intestinal mucosa for macromolecules is a well-established factor. In addition, the test was done in cases where positive results were expected on the basis of clinical, laboratory and X-ray findings [11, 18], and in control children. In each instance, total serum protein and its fractions were determined before and one hour after injecting the dye.

In 7 children with nephrosis, Congo red was used 11 times; the results were positive in each case. The intensity of faecal staining varied from mere traces (a control after distinct improvement) to a massive red colour. A particularly intensive red colour was observed in two children in whom the experiment was repeated after some weeks in order to confirm the success of therapy. One case was tested three times. This child and two others with nephrosis received 100 ml of 20 per cent human albumin immediately after the Congo red had been injected. According to the intensiy of the stools' colour a great amount of the dye must have escaped into the intestines. This observation is of considerable importance, as it is capable of informing us of the utilization of proteins and of the fate of PVP and dextran, and therefore of their therapeutic effect. It seems noteworthy that in children with nephrosis at the time when the

greatest amounts of protein are lost in the urine, the intestinal dye losses are less intensive and they become more significant later on, when the urinary losses are decreasing. These findings are in accordance with those of KLUTHE et al. [15, 16], and with the fact that the plasma protein level sometimes tends to decrease, even after urine losses have nearly ceased. LEAHY [17] on the other hand denies the occurrence of intestinal protein loss and is of the opinion that if such can be observed, it is caused by corticosteroid therapy.

Of our patients with nephritis only two acute cases gave a positive test, probably owing to a general capillary toxicosis. In cases of chronic nephritis, we found no dye in either the urine or the stools.

Six cases of coeliac disease yielded positive results without exception. The stools of three of these patients were stained intensively shortly after a crisis. A control test in one of the patients who then was symptomless and receiving a gluten-free diet, resulted in dye-free stools, thus providing a reliable indication of the therapeutic effect. In cases where the syndrome was due to other factors, e.g. giardiasis or hypersensitivity to milk, the positivity disappeared when treatment was successful. One dubious case was found to be negative. A case of suspected terminal ileitis was strongly positive, in 1 hour 96 per cent of the injected dye had disappeared from the blood stream.

In a case of congenital megacolon only traces of dye were found in the stools, and the result was negative on repeating the test. In three other children with megacolon the stools were negative, even after administering 80 ml of plasma immediately following the Congo red. Similarly, five children with gastroenteritis and pathological X-ray findings gave a negative test, only one with dyspepsia was strongly positive. According to MARSHAK [18] intestinal X-ray changes may precede protein losses.

Two patients with congenital septal defect were strongly positive. Traces of dye were only found in two of seven other cases of congenital heart disease, perhaps because all these children were well compensated and showing no cyanosis.

Each patient must undergo an exact clinical examination and a careful history should be taken. Both these facts are as important as a laboratory examination of the patient's blood protein level. In one of our patients, suffering from sarcoidosis, even hypergammaglobulinaemia was found. This boy had not been gaining weight for some time, in spite of a good appetite and adequate food intake. The Congo red test showed severe dye loss into the intestine. A similar case was observed in a girl with Virchow's nanosomia whose originally positive stools became negative after improvement had set in.

The same was the course of events in a patient with amelia while the test was repeatedly negative in two amelic siblings. A positive result was yielded by a child who had a streptococcal infection shortly before

the test. A control test, performed in the same child in full health, was completely negative. These results make it seem that the permeability of the intestinal mucosa for blood proteins is by no means a constant factor, but is influenced by the general state of health of the patient.

The test was positive in two siblings with liver cirrhosis, one child suffering from diabetes insipidus and one with sclerodermia. Two further children with allergic eczema were also tested. In the one, whose disease was in a state of remission, the stools were stained slightly, whereas in the other, examined in the acute phase, the result was positive. One asthmatic patient and one with thrombopenic purpura had no dye in the stools. The test should be indicated carefully in patients of this kind, though Congo red is supposed to be anti-allergic.

Positive results were also seen in two infants with severe congenital malformations of numerous organs. One of them had also hypogammaglobulinaemia, the other pathological X-ray findings in the digestive tract. One case each of Hodgkin's disease, Zollinger—Ellison's syndrome and Milroy's disease had coloured stools.

In general, the Congo red test is simple to perform and practically without any danger. We have not met with any serious complications, although in three cases slight cyanosis and dyspnoea were noted but then the Congo red was not freshly prepared. A disadvantage of the test is its non-quantitative aspect. We are endeavouring to work out a method which would allow to determine the amount of dye in the faeces. Congo red being soluble in acid acetone and, in addition, fluorescing [20], the task may not be too difficult. If the problem could be solved, the test were of the same value as those using radioactive substances and it would make it possible to determine and even localize lesions of the intestinal mucosa of anatomical, toxic or infectious origin, and enable one to evaluate conservative and surgical therapeutic effects.

Summary

The fact that certain dyes are readily bound by albumin has been made use of in establishing protein losses into the digestive tract. Congo red was injected by the intravenous route and its appearance in the stools was studied. The test was performed on 91 occasions in 82 children. The results were in good agreement with the results of other methods (immune-electrophoresis, use of radioactive substances, etc.). The advantages and disadvantages of the method have been discussed.

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Dr. O. Saxl
Kunzova 8
Brno, Czechoslovakia