

From Medical Department F, Glostrup Hospital,
Copenhagen, Denmark.

STUDIES ON ANTIBODY PRODUCTION IN URAEMIA

By

INGER MARIE BALLING & MICHAEL SCHWARTZ

The reason why homotransplantation is so difficult is that Nature really never meant such a procedure to be successful.

If it is attempted in spite of all, a defence mechanism of immunological character sets in, based upon so-called delayed hypersensitivity, *i.e.* cellular antibodies produced by lymphocytes. As a result, the graft is rejected.

In certain situations, however, homotransplantation does prove successful—or else the rejection is highly delayed, perhaps infinitely:

- (1) in cases of full compatibility (e.g. monozygotic twins),
- (2) in avascular tissue (cornea—cartilage),
- (3) in special sites: the anterior chamber of the eye, the brain, and finally the well-known situation of a foetus *in utero* where it is allowed to remain for 9 months,
- (4) in certain *non-specific* experimental procedures: During corticosteroid therapy, during treatment with antimetabolites, and following heavy X-ray irradiation. As for *specific* experimental procedures for possibly avoiding rejection of a homograft, there seems to be only one, viz. antilymphocyte serum,
- (5) in certain disease states: sarcoidosis, Hodgkin's disease, and last perhaps in uraemia.

HOMOTRANSPLANTATION IN URAEMIA

Little has been done in the way of studying the immunological conditions in uraemia. This is rather surprising, since the very fact that renal transplantation has proved possible must lead to the assumption that uraemic patients have special changes in immunological capability which to some extent counteract the rejection of the homograft. Only very few studies have been done in this field.

Dammin et al., in 1957 (1), investigated six uraemic patients on whom they tried *skin grafting*. The skin grafts remained *in situ* for a very long time, up to 115 days, before signs of rejection were manifest, while normally they are rejected in a few days.

Antibody production in uraemic patients has been studied by few workers. *Stoloff* and his associates (4) investigated the synthesis of diphtheria antitoxin in 14 uraemic patients. They found the antitoxin production to be of exactly the same magnitude as in a normal material. However, it must be mentioned that the uraemia was not particularly severe in all cases.

A comprehensive study was carried out by *Wilson* and associates (5, 6). More or less indirectly, they investigated the delayed as well as the immediate hypersensitivity in uraemia in a large number of patients from Starzl's material in Denver. The investigations were published in 1964 (5) and 1965 (6). Both publications cover approximately the same patient materials, and nearly the same technique was used. The latter study (1965) was supplemented by microscopic examinations of excised thymus glands and lymphocyte countings.

To study *delayed reaction* *Wilson et al.* used 7 antigens: tuberculin, histoplasmin, blastomycin, coccidiomycin, mumps skin test antigen, and *Candida* as well as *Trichophyton* antigen.

With these antigens 33 normal and 33 uraemic patients were examined, *i.e.* an intracutaneous test was done and the

results read at the end of 24-48 hours. The 33 normal persons showed a total of 89 positive reactions as compared with 28 positive cutaneous reactions in the 33 uraemic patients. Thus, the uraemic patients had at least less antibody content in the skin. With typhoid toxoid they studied the *production of circulating antibody* in 10 uraemic patients and in 33 normals. The uraemic patients showed a distinctly reduced antibody production after O as well as H antigen.

A number of *immediate skin reactions* were studied in 42 uraemic patients and 42 normal persons using 6 different extracts, partly of fungi and partly of various pollens. 37 positive cutaneous reactions were observed in the uraemic patients and 104 in the normals (who were, incidentally, potential kidney donors for the uraemic patients).

To investigate the *reactivity of the skin*, 14 normal persons and 14 uraemic patients were injected intracutaneously with histamine. This showed no difference between uraemic patients and normals.

Renal transplantation was performed on 23 of the uraemic patients, and the cutaneous reactions were tested again after the operation. Out of 61 cutaneous reactions in uraemic patients that had been negative prior to the operation 47, or 77 per cent, had now become positive. This must be due to transfer of antibodies (with lymphocytes) from the transplanted kidney.

Another investigation of 25 patients provided with a new kidney correlated the cutaneous reactions prior to the renal transplantation with a possible rejection reaction and the time of this reaction. One patient had negative cutaneous reactions and exhibited no rejection reaction at all. 13 patients, also with negative cutaneous reactions, showed signs of rejection an average of 14.8 days after the transplantation. 11 patients who had shown one or more positive cutaneous reactions before the operation exhibited signs of rejection, but after an average of only 4.3 days.

By these studies *Wilson et al.* have clearly shown that (1) in uraemia the immunological response is frequently sup-

pressed and that (2) this weak immunological response is evidently an advantage to the uraemic patient when he is to have a renal transplantation.

Conversely, it must be justified to conclude that if the antibody response in a uraemic patient can be measured, possible treatment to avoid rejection should be most intensive in those patients who show the strongest antibody production.

PRESENT INVESTIGATION

With the assistance of several medial departments in Copenhagen we tried to study the antibody production in uraemic patients.

The antigen used was dinitrochlorobenzene (DNCB). After suitable exposure, anyone can be sensitized to this substance. The reaction is of a typical delayed type, and the onset of sensitization can be accurately measured by an eczematous reaction which is very simple to read. By using suitable amounts of DNCB it is possible to sensitize a predictable number of persons. The reaction is entirely specific and, as already mentioned, it represents cellular hypersensitivity of the same type as the rejection reaction.

The procedure of sensitizing the normal persons and the patients was the same as previously described (3). A 1 per cent solution of DNCB in acetone was prepared, and for the sensitization 0.1 ml of this solution (1 mg DNCB) was dropped on the skin over an area of 3.3 square cm. The site was inspected at the end of 28 days, and if a positive reaction was present, in the form of eczema, this was recorded. In the event of a negative reaction, 0.3 ml of a 0.1 per cent solution of DNCB ($30\mu\text{g}$) was dropped on a new cutaneous site. At the end of 4 days the reaction was read. Persons who were still negative were again tested with 0.3 ml of a 1 per cent solution of DNCB ($300\mu\text{g}$) on a third cutaneous site. At the end of 28 days positive and negative reactors were recorded. All those, who showed a positive reaction with eczema at the site of testing after one of the three exposures and at the final

reading 28 days after the last exposure, were recorded as being sensitized to DNCB. In the above-mentioned previous study (3) sensitization was found in approximately 50 per cent of the normal population.

The results of studying the uraemic patients and the normals are shown in Table 1. It will be seen that 61 per cent of the 63 normals, but only 32 per cent of the 50 uraemic patients were sensitized. This difference is statistically significant ($p < 0.01$).

TABLE 1
*Antibody production after exposure to DNCB in normal persons
and in uraemic patients.*

	N	+ antibody	% pos.
Normals	63	39	61
Uraemic patients	50	16	32

In Table 2 the degree of sensitization is correlated with the serum creatinine concentration. From very slight sensitization in the patients showing the most severe uraemia there is an even increase to the milder cases.

In other words, the results quite clearly show that patients with uraemia—as might be assumed from previous studies—are poor antibody producers, the poorer the more severe the uraemia.

TABLE 2
*Antibody production after exposure to DNCB in normal persons
and in uraemic patients.*

	Se creatinine (mg/l)	N	+ antibody	% pos.
Uraemic patients	≥ 100	12	2	16
	$< 100 \geq 60$	11	4	36
	< 60	27	11	41
Normals		63	39	61

This method of testing with DNCB is in many respects well-suited for studying the power of sensitization in a group of patients. DNCB is chemically well-defined, and the reaction elicited is simple and very easy to read. Apparently there are no cross reactions, and as a rule people do not come across the substance later; this also accords with the fact that „spontaneous“ sensitization to DNCB hardly ever occurs.

Perhaps the 50 per cent sensitization among the normals is too low. May be it is possible, by increasing the dose of dinitrochlorobenzene, to obtain better separation of the very poor, rather poor, and fairly good antibody producers among uraemic patients.

DISCUSSION

On the basis of the studies available so far there is no doubt that the ability to respond to immunological stimulation is greatly suppressed in patients with chronic uraemia. This reduced reactivity seems to comprise the so-called delayed hypersensitivity as well as the allergic reaction of the immediate hypersensitivity type.

Nothing definite is known about the pathogenesis of this suppressed antibody production. We know that foreign antibody, transferred to uraemic patients by lymphocytes from a donor, functions normally (see above). We also know that the skin of these patients responds normally to histamine. It must be assumed, therefore, that the immunological defect in uraemia affects the antibody production proper. It has been suggested that this defect might consist in a generally reduced synthesis of protein, a reaction which is presumably responsible for the anaemia so well-known in uraemia (*Wilson et al.* (5)).

As far as the formed elements of the blood are concerned, we know that the granulocyte count is normal or increased in uraemia (2) and that the platelet (and megakaryocyte) count is also normal. On the other hand, red-cell production is reduced (anaemia), and there is also a reduced number of

circulating lymphocytes. *Wilson et al.* (6) found that while the circulating lymphocytes averaged 2700 per μ l in normals, the lymphocyte count in their uraemic patients was less than half: 1100 per μ l. This difference is statistically significant.

Another factor too may be operative in uraemic patients. As already mentioned, some of the uraemic patients (11 men) were thymectomized prior to the renal transplantation. On microscopic examination, all the thymic glands showed major or minor degrees of atrophy. Although it may be difficult to interpret the relationship between this thymic atrophy and the reduced immunological response in uraemic patients, it might be imagined that the thymus has its functions in antibody production beyond the first weeks of life and thus may be more or less responsible for the suppressed antibody synthesis in uraemia.

SUMMARY

In sensitization experiments using dinitrochlorobenze normal persons showed a sensitization rate of 63 per cent, while in uraemic patients the sensitization rate was only 32 per cent, and in severe uraemia only 16 per cent.

It is probable that this reduced reactivity comprises delayed as well as immediate hypersensitivity.

REFERENCES

1. *Dammin, G. J., Couch, N. P. & Murray, J. E.*: Ann. N. Y. Acad. Sci. 64, 967, 1957.
2. *Riis, P. & Stougaard, I.*: Dan. Med. Bull. 6, 85, 1959.
3. *Schwartz, M.*: J. Allergy 24, 143, 1953.
4. *Stoloff, I. L., Stout, R., Myerson, R. M. & Havens, W. P.*: New Engl. J. Med. 259, 320, 1958.
5. *Wilson, W. E. C. & Kirkpatrick, C. H.*: In Starzl, T. E.: Experience in renal transplantation, Saunders, Philadelphia, 1964 p. 239, pp. 383.
6. *Wilson, W. E. C., Kirkpatrick, C. H. & D. W. Talmadge*: Ann. Int. Med. 62, 1, 1965.