Do Schwann cells produce collagen type III?

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Summary. The fact that collagen from both normal nerve endoneurium and Schwann cell tumours present characteristics of collagen type III, suggests that Schwann cells produce this type of collagen.

The participation of Schwann cells in the production of collagen in nerves has been a matter of controversy. Results obtained studying nerve regeneration[1] suggest that these cells produce collagen. The fact that pure Schwann cell cultures produce thin fibrils, morphologically similar to collagen, and can also incorporate proline into protein and hydroxylate this aminoacid[2,3] strongly supports the above contention.

Recently the biochemical study of human femoral nerves[4] demonstrated the presence of collagen types I and III in these structures. The distribution of these types of collagen in the peripheral nervous system has, however, not been reported so far.

To study the localization of collagen types I and III in nerves, we applied the method, developed in this laboratory, that permits the distinction of these collagen types in tissue sections by means of optical microscopy[5]. This method is based on the observation that in tissue sections, regions known to contain collagen type III, present thin pale green fibres when previously stained with Sirius Red and studied with polarization microscopy; while collagen type I – under the same conditions – presents thick fibres strongly coloured in yellow or red.

When studied by this method, all peripheral nerves obtained from 14 representative species of vertebrates (comprising fish, amphibia, reptiles, birds and mammals, including man) showed similar results characterized by the abundance of pale green thin fibres observed in the perineurium and endoneurium contrasting with the thicker yellow or red fibres of the epineurium. These results strongly suggest that collagen type I is present in the connective tissue of the nerve sheaths, while type III is mainly in the endoneurium.

The fact that the endoneurium is separated from the epi- and perineurium by the so-called perineural epithelium which constitutes not only a morphological but also a biochemical and biological barrier, allied to the observation that fibroblasts are very scarce in the endoneurium[6], lead us to believe that the Schwann cells are the most probable candidate for the site of collagen type III synthesis in nerves.

These results receive support from unpublished observations from this laboratory, showing that in tumours derived from Schwann cells (Schwannomas or neurinomas)[7], with exception of peripheral strands of collagen type I belonging to the tumour's capsule, all collagen fibres present characteristics of collagen type III.

It would be interesting to check these results with the immunofluorescent methods developed recently for the tissue localization of collagen types I and III[8], and also quantitate, biochemically, the ratio of collagen types in Schwannomas.

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The influence of homologous plasma and fetal calf serum on human lymphocytic cortisol metabolism[1]

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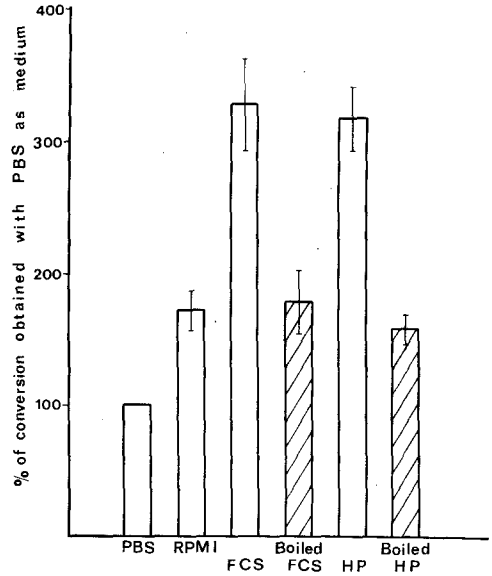
Summary. The influence of a) tissue culture medium (RPMI), b) homologous plasma (HP), and c) fetal calf serum (FCS) on lymphocytic cortisol metabolism was compared to that of phosphate buffered saline alone. RPMI was found to enhance the conversion rate 1.71 times, whereas HP and FCS enhanced it about 3.2 times. Raising the temperature of the HP and FCS to 100 °C before incubation reduced the enhancing effect to the level of that obtained with RPMI.

The destructive effect of cortisol on lymphocytes, as well as the ability of lymphocytes to effect alterations in the molecular structure of cortisol, have been demonstrated by Dougherty and his associates[3,4]. These workers have postulated that the ability of lymphocytes to effect changes in cortisol constitutes an important homeostatic mechanism in the regulation of the lymphocyte population. Studies by Jenkins[5], who used tissue culture media, and by Klein et al.[6,7] showed that human lymphocytes are capable of metabolizing cortisol to tetrahydrocortisol (3α, 11β, 17α, 21-tetrahydroxy-5-pregnan-20-one), 20α-hydroxycortisol (11β, 17α, 20α, 21-tetrahydroxy-4-pregnene-3-one), and 20β-hydroxycortisol (11β, 17α, 20β, 21-tetrahydroxy-4-pregnene-3-one). To the best of our knowledge, no attempt has yet been made to investigate the influence of plasma on lymphocytic cortisol metabolism. In the present study, the influence of tissue culture medium (RPMI), homologous plasma (HP) and fetal calf serum (FCS) (the latter 2 both

boiled and unboiled) on human lymphocytic cortisol metabolism was investigated and the results compared to those obtained when only PBS was used as medium.

Material and methods. Lymphocytes were prepared from buffy coats obtained from the blood bank. They were isolated by centrifugation at 400 × g using the Ficol-Isopac method, washed twice and resuspended in PBS (pH 7.4) containing penicillin and streptomycin, 100 IU/ml each. Each preparation of lymphocytes was divided into series of flasks so that each flask contained an average of 88.10^6 lymphocytes in 1 ml suspension, the size of the series being determined by the number of lymphocytes obtained. 1 ml of a) PBS, b) RPMI, c) HP, d) FCS, e) boiled HP, or f) boiled FCS (plasma or FCS were heated on an open flame till boiling) were than added to the flasks. In addition to the lymphocytes and various media each flask contained a) NADPH generating system composed of 1.0 µmole NADPH, 5.0 µmoles glucose-6-phosphate, 1.0 Korenberg unit of glucose-6-phosphate dehydrogenase and 1.4 µmoles $MgCl_2$, all dissolved in 0.1 ml PBS, and b) 50 µg unlabelled cortisol + 5.0 µCi 1.2-^{3}H-cortisol (final S.A 36.2 mCi/mmole). A blank containing all the components with the exception of the lymphocytes was also processed. The sealed flasks were incubated in a shaking bath at 37 °C for 17 h. At the end of the incubation period the contents of each flask were extracted twice with 5 ml chloroform and evaporated to dryness under nitrogen. The residue was dissolved in ethanol and applied on silica gel HF-254 thin-layer plates. The plates were developed in chloroform: methanol (90/10 v/v)[8]. Following chromatography the plates were reviewed in UV-light at 254 nm and scanned using a radioactivity scanner. The product and substrate spots as well as the remainder of the plate (for recovery calculations) were scraped off and extracted with ethanol (final volume 5.0 ml). Samples of 0.1 ml were transferred into scintillation vials and the radioactivity read in a liquid scintillation spectrometer.



The effect of various culture media on lymphocyte cortisol metabolism. The level of metabolism in the presence of PBS is taken as 100% reference. The conversion rates of all the various media were significantly increased as compared to PBS (HP and FCS, $p < 0.001$; RPMI and boiled HP, $p < 0.05$; boiled FCS, $p < 0.02$). There were no significant differences when the relative conversion rates of the following were compared: a) HP and FCS, b) boiled FCS and boiled HP, and c) the 2 latter compared with RPMI. Raising the temperature of HP and FCS to 100 °C before the start of incubation resulted in a significant decrease in cortisol metabolism when compared with that achieved with the identical media ($p < 0.001$ and $p < 0.01$ respectively). PBS, phosphate buffered saline; FCS, fetal calf serum; HP, homologous plasma.

Results and discussion. Upon scanning the TLC plates, all of the extracts of lymphocyte suspensions showed 2 radioactive peaks, a major, less polar peak corresponding to cortisol itself, and a minor, more polar peak, representing the total amount of metabolized cortisol. The percentage of radioactivity of this peak calculated from the radioactivity of the added cortisol after recovery calculations was determined as the conversion rate. The conversion obtained with human lymphocytes in PBS was regarded as 100% and all the conversion rates obtained with the other media used were compared with it (figure). As can be seen, the conversion rates of all the various media were significantly increased as compared to PBS (HP and FCS, $p < 0.001$; RPMI and boiled HP, $p < 0.05$; boiled FCS, $p < 0.02$). There were no significant differences when the relative conversion rates of the following were compared: a) HP and FCS, b) boiled HP and boiled FCS, and c) the 2 latter with RPMI. Raising the temperature of HP and FCS to 100 °C before the start of incubation resulted in a significant decrease in cortisol metabolism when compared with that achieved with the identical unboiled media ($p < 0.001$ and $p < 0.01$ respectively).

These results could not be attributed to death of cells, since 95% viability was proved at the end of the incubation.

The fact that the enhancing effect of HP and FCS upon lymphocytic cortisol metabolism is temperature-dependent, but the conversion rate following boiling does not fall to that obtained with PBS, probably indicates that there are at least 2 factors responsible for the rise in activity. 1 of these factors is temperature-labile, probably a polypeptide, whereas the 2nd is temperature-stable. The fact that the increase in the conversion rate resulting from RPMI was similar to that obtained with boiled HP and boiled FCS, may be related to the presence of a common component in the three media. On the other hand, the temperature-labile factor demonstrated to be present in both the HP and FCS may differ in these 2 media, since the FCS contains proteins foreign to human lymphocytes, indicating a possible relationship between the change in the rate of lymphocytic cortisol metabolism and the immune response. This is a point which remains to be clarified, but it would appear that the rise in activity obtained with HP is due to a factor which enhances lymphocytic cortisol metabolism. Further to the findings of Dougherty and his co-workers, the study carried out by us demonstrates that plasma, whether homologous or heterologous, also plays an important role in the defense mechanism of lymphocytes against cortisol which is postulated to be present in the regulation of lymphocyte population.

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