## The Acute Inflammatory Response in Experimentally-Induced Pleural Edema

The inflammatory response induced in the rat by intrapleural injection of a 0.1% solution of Evan's blue dye was described first by I. MERITS, who studied the changes in composition of the resulting edema fluid over a 24 h period[1]. The method was adapted by HOLTKAMP et al. for the short-term (6 h) assay of potential anti-inflammatory agents[2]. LADEN et al.[3] and SARKER and FOSDICK[4] also have used the technique for their studies of inflammation. The earliest that the pleural fluid volume and/or its composition was determined by these investigators was 3 h after dye injection[1].

This paper reports the acute changes in fluid volume and the accumulation of plasma proteins observed during a 6 h period after injection of the dye solution.

*Procedure.* Male rats (Sprague-Dawley strain) weighing 300 ± 20 g were divided into groups of 6. To determine the increase in capillary permeability due to pleural inflammation, either 0.9% saline (controls) or 0.5 μc of radioiodinated serum albumin (RISA) in 0.1 ml of saline was injected via the tail vein. Ninety min later 5 ml of 0.1% Evan's blue dye solution (at 37 °C) was injected into the pleural cavities of the rats at the level of the fifth rib. The animals were killed at intervals so that there was a group of 5 RISA-injected and 1 saline-injected rats for 0.5, 1, 2, 3, 4, 5 and 6 h after dye injection. Blood was collected from severed neck vessels in a heparinized vial provided with a heparinized funnel. The pleural fluid was removed from the exposed pleural cavity and its volume measured by the use of the suction device shown in Figure 1, and then it also was poured into a heparinized vial.

To compensate for unequal sample volumes of pleural fluid and blood for determination of radioactivity, a 2 ml aliquot of each was pipeted into a clean heparinized vial and the radioactivity level was measured using a scintillation counter. The samples from the saline-injected rats acted as background controls. (The detection system was composed of a Nuclear Chicago Model 183 scaler and a Nuclear Chicago Model DS-3 scintillation well detector with a sodium iodide thallium-activated crystal possessing an $I^{131}$ efficiency of 51%.) The data were recorded as the means of the ratio counts/min (cpm) pleural fluid/cpm blood for each animal in a group.

*Results.* The results of 3 studies to determine the influence of time on the pleural fluid volume are presented in Table I. They show that within 0.5 h the volume had decreased markedly and continued to decline to a minimum at 3 h. The volume changed little during the fourth hour, but there was a sharp increase in volume during the fifth hour. The volume continued to increase during the sixth hour, but it never equalled the injected dye volume. Others have shown that the volume will continue to increase and eventually will exceed the injected dye volume[3,4].

Table II presents the data from 2 studies of the movement of RISA from the peripheral circulation into the pleural fluid. There was an initial rise in the radioactivity of the pleural fluid which extended into the second hour. This was followed by an apparently smaller increase in activity between the fourth and fifth hours which coincided with the increase in the fluid volume as shown in Table I. These data are considered in more detail in the discussion (see also Figure 2).

*Discussion.* The conclusion of an earlier investigator that 'the volume of fluid in the pleural cavity remained almost constant up to 18 h'[1] is not consistent with the data presented here or elsewhere[2]. Our experiments showed a rapid fluid loss for the first 2 h and the volume

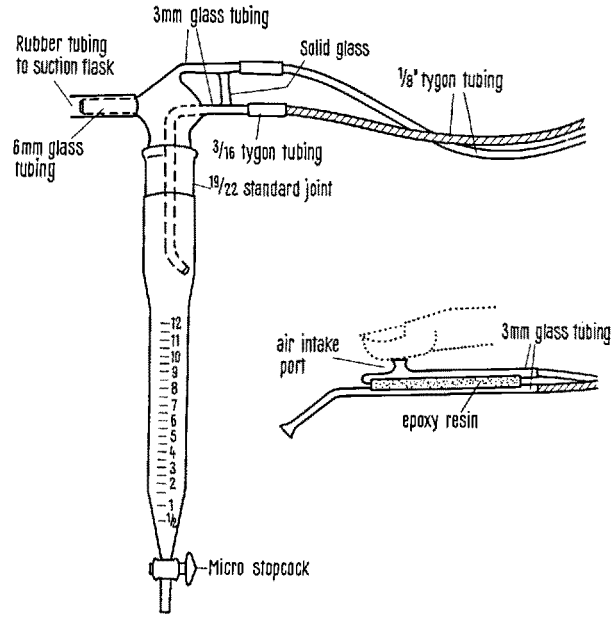


Fig. 1. Apparatus used to remove and measure pleural fluid volume.

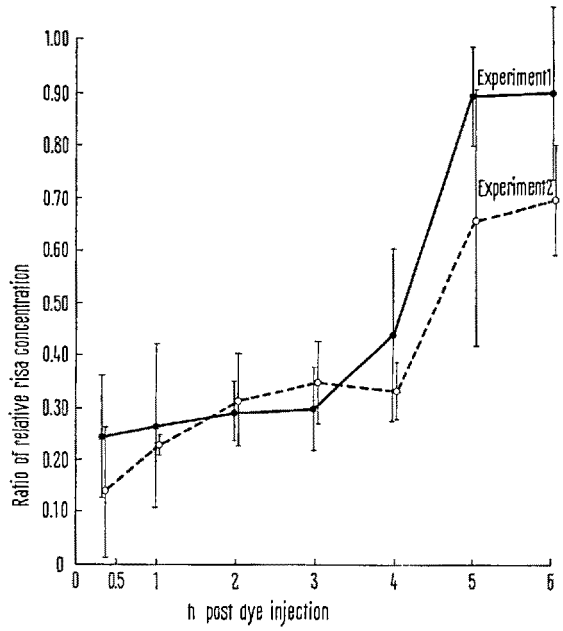


Fig. 2. Change in ratio of relative RISA concentration with time.

[1] I. MERITS, *Chemical Studies of Inflammatory Edema, Experimentally Induced* (Northwestern University Ph.D. Thesis, Evanston, Illinois 1955).

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[4] N. SARKAR and L. S. FOSDICK, J. Pharmac. exp. Ther. *146*, 258 (1964).

Table I. Variations in pleural fluid volume with time

| Experiment[a] | Intrapleural fluid volume (ml), mean ± S.D. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 30 min | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| 1 | 3.3 ± 0.5 (6)[b] | 3.1 ± 0.6 (5) | 2.8 ± 0.3 (5) | 2.5 ± 0.5 (5) | 2.8 ± 0.4 (6) | 3.8 ± 0.3 (6) | 4.2 ± 0.2 (6) |
| 2 | 3.5 ± 0.4 (6) | 3.3 ± 0.7 (5) | 2.8 ± 0.3 (6) | 2.6 ± 0.4 (6) | 2.8 ± 0.6 (6) | 3.8 ± 0.3 (6) | 4.2 ± 0.1 (5) |
| 3 | 3.2 ± 0.2 (5) | 3.0 ± 0.1 (6) | 2.7 ± 0.2 (5) | 2.6 ± 0.3 (6) | 2.6 ± 0.3 (6) | 3.9 ± 0.4 (5) | 4.0 ± 0.5 (6) |
| Combined | 3.3 ± 0.5 (17) | 3.1 ± 0.5 (16) | 2.8 ± 0.3 (16) | 2.6 ± 0.3 (17) | 2.7 ± 0.4 (18) | 3.8 ± 0.3 (17) | 4.1 ± 0.3 (17) |
| % change | 34% | 38% | 44% | 48% | 46% | 24% | 18% |

[a] These 3 experiments were run within a 5 week period. [b] No. of animals.

Table II. Movement of radioiodinated serum albumin (RISA) from blood into pleural fluid

| Experiment | Ratio of cpm of RISA/ml pleural fluid:cpm of Risa/ml blood | | | | | | |
|---|---|---|---|---|---|---|---|
| | 30 min | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| 1 | 0.336[a] ± 0.120 (5)[b] | 0.378 ± 0.125 (4) | 0.507 ± 0.087 (5) | 0.556 ± 0.082 (5) | 0.768 ± 0.146 (5) | 1.168 ± 0.103 (5) | 1.079 ± 0.191 (5) |
| 2 | 0.296 ± 0.157 (5) | 0.372 ± 0.029 (5) | 0.562 ± 0.138 (4) | 0.678 ± 0.104 (5) | 0.661 ± 0.130 (5) | 0.830 ± 0.229 (4) | 0.908 ± 0.188 (5) |
| Combined | 0.316 ± 0.133[c] (10) | 0.375 ± 0.079 (9) | 0.531 ± 0.109 (9) | 0.617 ± 0.109 (10) | 0.715 ± 0.150 (10) | 1.017 ± 0.238 (9) | 0.993 ± 0.200 (10) |

[a] Mean value for experiment. [b] No. of animals. [c] Mean ± standard deviation for combined data.

remained at a reduced level from the second to the fourth hour. The passage of this fluid was accomplished via the lymphatic and blood capillaries[5]. We propose that this initial reduction in fluid volume contributed to the elaboration of the inflammatory response by producing an increased concentration of Evan's blue dye. If one assumes that all of the dye remained within the pleural cavity and in solution, the relative amount was increased from 1 mg/ml to 1.5 mg/ml at the end of 0.5 h and to 1.9 mg/ml by the end of 3 h[6]. This concentration of the dye accentuated its mildly irritating effect and thus helped precipitate an inflammatory response.

The inflammatory response is characterized by a local dilation of blood vessels and increased permeability of the capillary endothelium[7]. The increased endothelial permeability permits diffusion of plasma proteins into the surrounding tissues. At the same time the volume of fluid diffusing from the vascular system exceeds that diffusing into it from the interstitial spaces, due in part to the increased interstitial colloid osmotic pressure resulting from protein leakage. Progressive fluid accumulation in the interstitial spaces ensues until a new equilibrium of fluid movement becomes established.

In the studies presented, the fluid diffusion rate from the pleural cavity exceeded or equalled the diffusion rate into it during the first 4 h after dye injection, which suggests that accumulation of plasma protein in the pleural cavity had not occurred yet. However, the data (Table II) indicate an early rapid movement of protein into the cavity, but this accumulation of protein may be more apparent than real. One reason is that the initial blood and pleural fluid samples were not taken until 2 h after RISA injection, thus allowing time for an expected leakage of protein into the pleural space to occur[5]. Such protein is returned to the blood vascular system via the lymphatics, e.g. RISA has been detected in the thoracic duct of dogs 7 min after i.v. injection[8]. Another reason is the concentration of slower diffusing molecules, the result of rapid water loss during this period and the slow lymph flow rate from the pleural cavity[3] – a flow rate which normally would be adequate. Thus the establishment of an osmotic equilibrium between the pleural fluid and the blood was due in a greater degree to the movement of water out of the pleural cavity than the diffusion of protein into it.

One means of eliminating the influence of the decreasing pleural fluid volume on the ratio of relative RISA concentration is to assume that the volume of fluid in the pleural cavity remained at 5 ml throughout the test period and recalculate the data on the basis of this assumption using the following formula[9]. A plot

$$Y = \frac{(P/B) \cdot V_t}{V_i}$$

of the adjusted values is shown in Figure 2.

[5] C. H. BEST and N. B. TAYLOR, *The Physiological Basis of Medical Practice*, 7th edn (Williams and Wilkins Co., Baltimore 1961), p. 36.

[6] Assume a mean pleural fluid volume at 0.5 h of 3.3 ml and at 3 h of 2.6 ml compared to the 5 ml injected originally.

[7] W. G. SPECTOR and D. A. WILLOUGHBY, Bact. Rev. *27*, 117 (1963).

[8] K. WASSERMAN and H. S. MAYERSON, Am. J. Physiol. *165*, 15 (1951).

[9] Where $Y$ = adjusted value of ratio of relative RISA concentration, $P/B$ = ratio of relative RISA concentration, $P$ = cpm of RISA/ml of pleural fluid, $B$ = cpm of RISA/ml of blood, $V_t$ = volume of pleural fluid at time $t$, $V_i$ = volume of 0.1% Evan's blue dye injected = 5 ml.

The RISA data, as presented in Figure 2, tend to substantiate our thesis that the inflammatory response in the intrapleural edema test is not produced immediately upon injection of the dye solution but develops gradually. However, at the end of the fifth hour, an inflammatory response had developed fully as indicated by the elevated ratio of relative RISA concentration. This raised the intrapleural colloid osmotic pressure and a new diffusion equilibrium between the pleural cavity and the pleural vasculature then had to be established.

A leveling off of the volume and protein concentration of the pleural fluid would indicate establishment of a new equilibrium. The data for 6 h post dye injection show that the leveling off process had begun. It is significant that the concentration of RISA/ml pleural fluid had become almost equal to that in the blood (Table II) – a ratio of 1 would denote this. This suggests that almost complete mixing of plasma protein with interstitial protein had occurred[10].

*Zusammenfassung.* Untersuchungen der auffallendsten Veränderungen des pleuralen Exsudates nach experimentellem Pleuralödem bei der Ratte sowie über die Ausbreitung des RISA aus den Pleuralgefässen in die Intercostalhautflüssigkeit sprechen dafür, dass sich die entzündliche Reaktion besonders langsam entwickelt, da sie selbst 5 h nach Farbinjektion noch nicht sichtbar wurde.

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[10] We wish to thank Dr. M. FREE and J. PAULS of the Statistical Section for their advice and assistance in computing the statistical significance of the data. We wish to acknowledge the technical skill of J. BELZ in making the suction apparatus shown in Figure 1.

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## Lingually Induced Inhibition of Masseteric Motoneurones

The principal or main sensory nucleus of V is a compact nucleus of predominantly small cells which receives fine ascending branches of primary trigeminal afferents[1,2]. At its rostral extent it is bordered medially by the large cells of the trigeminal motor nucleus. In this area an extension of loosely arranged neurones from the main sensory nucleus caps the motor nucleus dorsally[1,3,4]. Some authors consider these cells to constitute a separate nucleus (e.g., the supratrigeminal nucleus of LORENTE DE NÓ)[1,4,5], while others describe them as the medial portion of the principal sensory nucleus[6]. Somatotopic analyses of single unit activity have shown that the buccal cavity is represented in this area[6,7]. It has been suggested on cytoarchitectural[4] and physiological[8] grounds that this region may contain an aggregation of interneurones active in trigeminal reflex pathways. To test this hypothesis we used the trigeminal reflex first described by MILLER and SHERRINGTON[3], in which lingual nerve stimulation produces reflex opening of the jaw in decerebrate cats.

A concentric bipolar stimulating electrode was inserted into the mesencephalic nucleus of V following cerebellectomy in the decerebrate, $C_2$ spinal sectioned cat. Animals were maintained under flaxedil and artificially respired. The ipsilateral masseteric nerve was dissected and cut distally; its central stump was placed on a bipolar silver wire electrode. This arrangement could be used either for recording the masseteric monosynaptic reflex induced by stimulation of the mesencephalic nucleus[9], or for the antidromic excitation of masseteric motoneurones in order to identify them during intracellular recording. The lingual nerve was sectioned bilaterally at the periphery and the central stumps were stimulated with a bipolar silver wire collar electrode. KCl or K-citrate filled micropipettes were used to record extracellular and intracellular potentials.

When low threshold afferent fibers in the ipsilateral lingual nerve were stimulated by a single pulse, a response in the form of a series of membrane potential changes were observed in masseteric motoneurones. A pulse of 10 μsec and 0.5–1.0 V could usually elicit this response, which was characterized by 3 phases: phase 1, hyperpolarization beginning with a latency of 2.3–2.9 msec with a peak at 8–10 msec; phase 2, depolarization with a peak at about 20 msec; phase 3, hyperpolarization peaking at about 40 msec followed by a gradual return to the prestimulus level (Figure 1A). These membrane potential changes corresponded closely to the changes in size of the masseteric reflex when tested following a conditioning lingual nerve stimulus (Figure 1B). The average size of the hyperpolarization in phase 1 was 5 mV. Antidromic and orthodromic spikes were readily blocked, and the membrane conductance was observed to increase during this phase. When recorded with KCl electrodes this hyperpolarization changed into a depolarizing potential with a similar time course to the original hyperpolarization. The evidence, thus, indicates that the hyperpolarization in phase 1 is an IPSP.

The degree of the return towards the control resting potential during phase 2 varied from cell to cell but in

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