# MACROPHAGE STIMULATING ACTIVITY OF MEATS AND ORGANS

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## SUMMARY

We screened macrophage stimulating activity in meats and organs. The nitrite formation was used as an index of the activity. Ten of the 38 water extracts from meats and organs had macrophage stimulants. Among the 10, chicken, cattle reticulorumen, swine stomach, chicken gizzard, swine cerebellum and swine tongue had higher activities. The amount of nitrite production by these stimulants was 30 to 70 nmol / $10^6$ cells.

## INTRODUCTION

Macrophages, when stimulated with lipopolysaccharide, produce a copious amount of intermediate nitric oxide from L-arginine through an enzymatic process (Marletta,1988; Iyenger et al.,1987), and the oxide then reacts spontaneously with molecular oxygen and water to yield nitrite and nitrate as final products (Marletta,1988; Stuehr et al.,1985). When reactive amino compounds co-exist in the system, N-nitroso compounds are also formed as final products (Miwa et al.,1989;1987). It has been proposed that nitritc oxide is one of the most important effectors for macrophage tumorcidal activity (Feldman et al., 1993, Hibbs et al., 1988,1987a,1987b). For this reason we have used nitrite formation as an idex of macrophage stimulation, and reported that macrophage stimulants existed in a wide variety of plants (Miwa et al., 1990). Here we report the results of screening for macrophage-stimulating activity in meats and organs.

## MATERIALS AND METHODS

Meats and organs. Meats and organs used in this experiment were prepared from cattle, swine and chicken which were slaughtered at National Institute of Animal Industry. After fat and connective tissue were removed, each of the materials was cut into small pieces. They were homogenized with an equal weight of water and centrifuged at 11,000 x g for 15 min. The supernatant was then freeze-dried. Ten water extracts containing macrophage

stimulants were dialyzed against distilled water to obtain their non-diffusible fractions. Each of the fractions was then freeeze-dried.

Cells and medium. A macrophage cell line (RAW 264.7) was obtained from the American Type Culture Collection. The cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (Bocknek Laboratories) at 37℃ in a humidified incubator containing 5% $CO_2$ and 95% air.

Nitrite formation assay. The freeze-dried samples were each dispersed in the supplemented MEM (Miwa et al.,1989) and stirred for 30 min. After centrifugation, the supernatant was sterilized by passage through a 0.22 $\mu$m filter. The cultured macrophages were removed from the dishes by vigorous pipetting and resuspended at a concentration of 1 x $10^6$ cells / ml. Cells were plated at 200 $\mu$l / well, allowed to adhere for 30 min, and then the medium was changed to the sample in the supplemented MEM. After 48 h of incubation, nitrite concentration in the culture supernatant was determined by a colorimetric method (Miwa et al.1989). Data were each expressed as an average of three independent measurements.

## RESULTS AND DISCUSSIONS

Table 1 shows macrophage stimulation activity in beef and cattle organs. Among 17 water extracts, only reticulorumen and cerebrum (20.0 and 2.0 mg / ml) stimulated macrophages to produce nitirte. Water extracts *per se* did not contain nitrite. The nitrite production by lipopolysaccharide (10$\mu$g/ml) was 66 nmol / $10^6$ cells. Table 2 shows macrophage stimulation activity in pork and swine organs. Six of 16 water extracts had the activity. Among the six, stomach and tongue had high activity. They stimulated macrophages at a concentration more than 2.0 mg / ml. The results in Table 3 shows that chicken and chicken gizzard had the macrophage stimulation activity. Chicken had the highest activity among the 38 materials investigated in this experiment and the nitrite production was 11.4 nmol / $10^6$ at a concentration of 0.2 mg / ml.

We (Miwa et al., 1990) have reported that a wide variety of plant foods stimulated macrophages, and 18 water extracts from 64 plant foods had the activity. The plant foods with higher nitrite producing activity generally contained non-diffusible fractions. In this experiment, we found that ten water extracts from three kinds of meats and 35 organs had macrophage stimulating activity. This ten extracts containing macrophage stimulants were dialyzed against distilled water. Table 4 shows that some of them lost stimulants by dialysis and the others contained non-diffusible stimulants. The non-diffusible stimulants

in swine cerebrum, swine cerebellum and chicken, chicken gizzard had higher activity than the activity before dialysis.

In our previous study (Miwa et al.,1990), we also reported that when a plant food contained stimulants, another one belonging to the same family had a high possibility of also containing stimulants. In this report, it is interesting that any of three stomach (cattle reticulorumen, swine stomach and chicken gizzard) had non-diffusible stimulants with high activity (Table 1-4).

Nitric oxide production in our body is positively controlled by a number of cytokines such as interferon-$\gamma$, tumor necrosis factor, interleukin-1 and interleukin-2. Since it is important whether the stimulants in meats and organs can controll nitric oxide prodution in our body, the characterization of the stimulants should be completly elucidated.

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